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ISSN 0038-3872

SOUTHERN CALIFORNIA ACADEMY OF SCIENCES

BULLETIN

Volume 118 **Number 2**

14

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10TH ST. AND CONSTITUTION AVE. NW
WASHINGTON DC 20013

118(2) 79-138 (2019) **August 2019**

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Date of this issue 16 September 2019

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Bull. Southern California Acad. Sci.
118(2), 2019, pp. 79–86
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Behavior of Young-of-the-Year Giant Sea Bass, *Stereolepis gigas*, off the Sandy Beaches of Southern California

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Abstract.—We made extensive observations in the shallows off sandy beaches along the southern California coastline from 2013-2018 using SCUBA. The common diurnal behavior of young-of-the-year (YOY) of giant sea bass, *Stereolepis gigas*, were documented, in detail, and categorized while they occupied their unique nursery areas. We identified and described the frequently observed behaviors referred to as 1) "kelping", 2) "resting", 3) "traveling", and 4) "burying". Finally, through direct observation, mysid shrimp were confirmed as the primary diet of young-of-the-year of the giant sea bass off southern California. This is the first study to provide a behavioral overview of the YOY of this internationally listed, endangered species, and it bridges important gaps in our understanding of their early life history.

Until recently, we knew little about the basic biology and life history of *Stereolepis gigas* because the over-exploitation of their fishery in the early 1900s depressed their populations and limited research (*c.f.* House et al. 2016). Documentation of the early life history of the ecologically and once economically-important giant sea bass is critical for both the successful management of that fishery and to provide a more complete baseline to aid with future studies of giant sea bass. Early developmental processes and recruitment patterns are crucial for completing the life history of any species, allowing us to make increasingly intelligent decisions about fisheries management policies and future conservation efforts, protect nursery sites, and encourage further studies of this species (Cailliet et al. 1996, Craig et al. 1999).

Quite the opposite of their rotund and dark adult counterparts, the young-of-the-year (YOY) giant sea bass are very small, terete but laterally compressed (perch-shaped), and depending on their age, their color phases transition through black, brown, then orange (Benseman and Allen 2018). Divers usually observe them as a bright orange or reddish fish with black and white spots, enlarged black pelvic fins, and transparent pectoral, caudal, and anal fins (Love 2011). The frequent low visibility in their preferred habitat, their unique shape, coloration, and behaviors that allow them to remain inconspicuous are adaptive, blending YOY giant sea bass into the surrounding detritus. These attributes, coupled with their preferred habitat of shallow, soft bottomed, and featureless flats just outside the wave base of sandy beaches (not frequented by divers) is perhaps why there were so few sightings of the YOY giant sea bass in the past. Using SCUBA transects, we were able to document for the first time the early ecology of the YOY giant sea bass *(c.f.* Benseman and Allen 2018).

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The specific goals of this contribution were to describe, in detail, the diurnal behavior that these YOY giant sea bass exhibit after recruitment and their use of those adaptations to facilitate predator avoidance and mobility under varying degrees of surge during the diurnal hours.

Materials and Methods

We chose five sites that yielded the highest probability of locating YOY giant sea bass for our behavioral observations (Benseman and Allen 2018). These locations included soft sandy beach bottoms areas near the heads of submarine canyons (Allen et al. 2018). These were Veterans Park in the City of Redondo Beach (33° 50′ 10.42″N, 118° 23′ 30.06″W), Newport Pier, off Newport Beach (33° 36′ 26.63″N, 117° 55′ 54.80″W), and La Jolla Shores in San Diego (32° 51′ 19.63″N, 117° 15′ 45.03″W) off southern California.

We amassed over 220 biologist hours of focused bottom time on SCUBA during the months when we expected YOY giant sea bass to be present, from July 2013 through February 2018. SCUBA observations were conducted during the daylight hours (0800-1700) at depths ranging from 2-18 meters. Diving observations were made about every 1-3 weeks at rotating locations as weather, schedules, and water conditions allowed. The first behavior seen at initial contact for each individual was recorded. We documented the behavior of 80 individuals on video with a GoPro© Hero 3 for 1-4 minutes.

Results

The diurnal behavior of YOY giant sea bass is yet another heretofore undescribed aspect of the early life history of the giant sea bass, so we separated the most frequently observed behaviors into the categories we referred to as 1) "kelping", 2) "resting", 3) "traveling", 4)"feeding", and 5) "burying". The three most common diurnal behaviors observed were "kelping" (Fig. 1), where the individual camouflages itself matching the form and movement of vegetation fragments, "resting" where the individual remained in place without accompanying vegetation or detritus, and "traveling" (Fig. 2), where the individual slowly moved 1 to 2 cm just above the sand. In Benseman and Allen (2018) and Couffer (2017), we documented three distinct color phases of YOY giant sea bass: black, brown, and orange, ranging from 10 to 185 mm TL and described the morphology of each phase in detail. Kelping behavior was the most frequent behavior encountered and was most commonly associated with the larger orange phase fish ($X^2 = 10.24$, $p = 0.03$; Fig. 3) which makes sense because they are the largest and most conspicuous of the three phases. As first described by Couffer and Benseman (2015), burying behavior was also witnessed, where an individual that was startled, turned on its side, undulated like a flatfish, and completely buried itself under the soft sandy bottom substrate (Fig. 4).

Kelping behavior (Fig. 1) undoubtedly functions as a predator avoidance mechanism, and perhaps secondarily, as an energy-saving behavior. YOY giant sea bass mimic scattered fragments of vegetation attached to the benthos or trapped against small-stationary objects such as solitary protruding worm tubes. Kelping always involved an individual orienting to a single isolated fragment or very small clump of algae. YOY giant sea bass appeared to prefer frond fragments of giant kelp, which are relatively common within the nursery areas, but they would also "kelp" against various other fragments of algae, small solitary tangles of dead surf grass, and in one instance, a piece of PVC tubing. White spots on the sides of the YOY of giant sea bass may mimic the white patches of bryozoans that are often sparsely

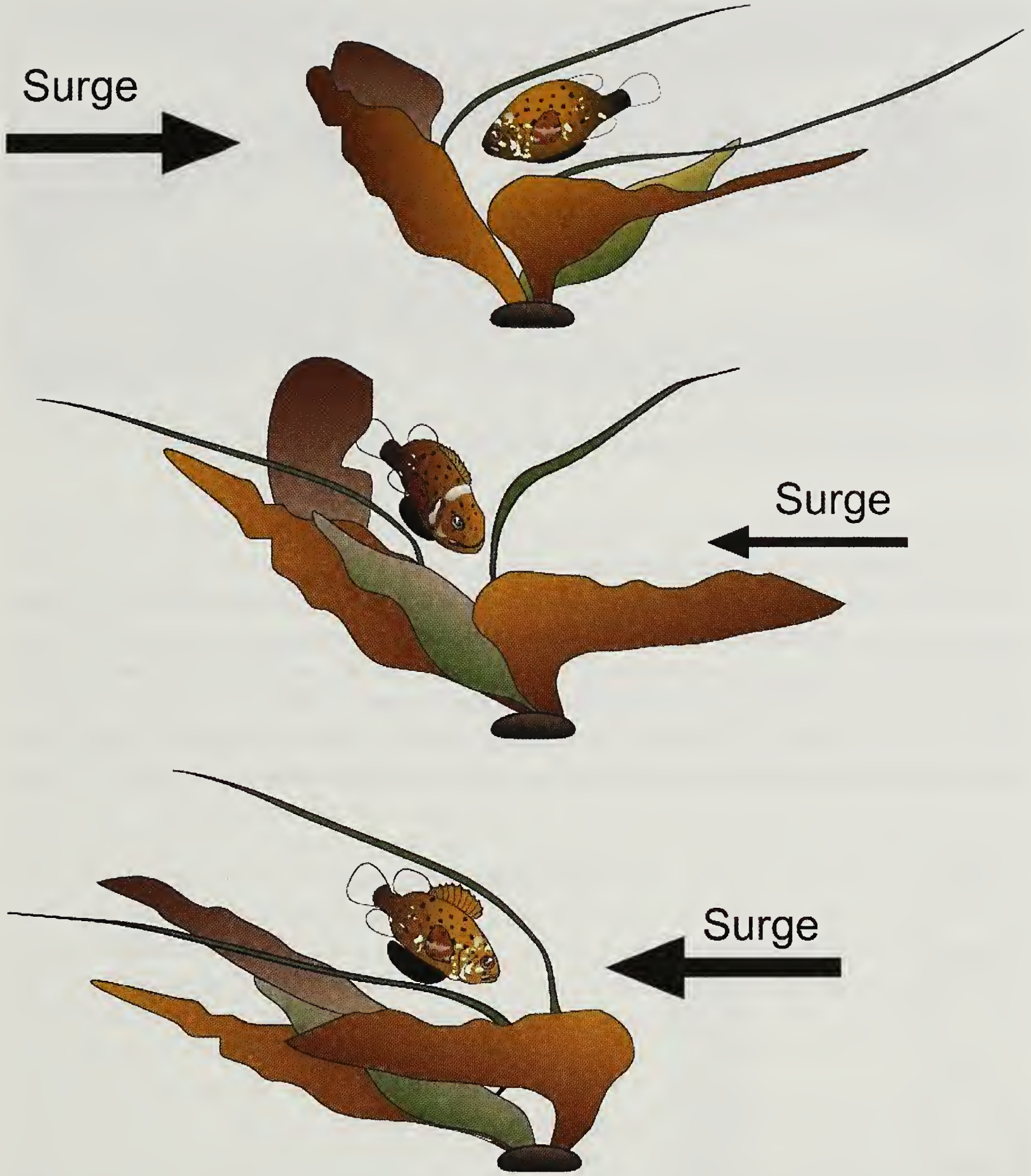

Fig. 1. Diagrammatic representation of "kelping" behavior with both vegetation and fish orienting to reversing surge. Drawn from video by LGA.

scattered across older kelp fronds and other objects. The fish usually stationed themselves next to a piece of kelp or detritus similar in size to the individual, and held position against the surge by swimming back and forth as the direction of surge changed, turning into the oncoming current and then back in the other direction as the surge direction switched (Fig. 1).

Contrary to earlier thought, YOY giant sea bass were not normally associated with sand dollar (*Dendraster excentricus*) beds. Out of 160 individuals observed during the 220 hours of observation, only two were observed in sand dollar beds. One was swimming across a bed, and the other was holding station next to a sand dollar. Moreover, YOY giant sea

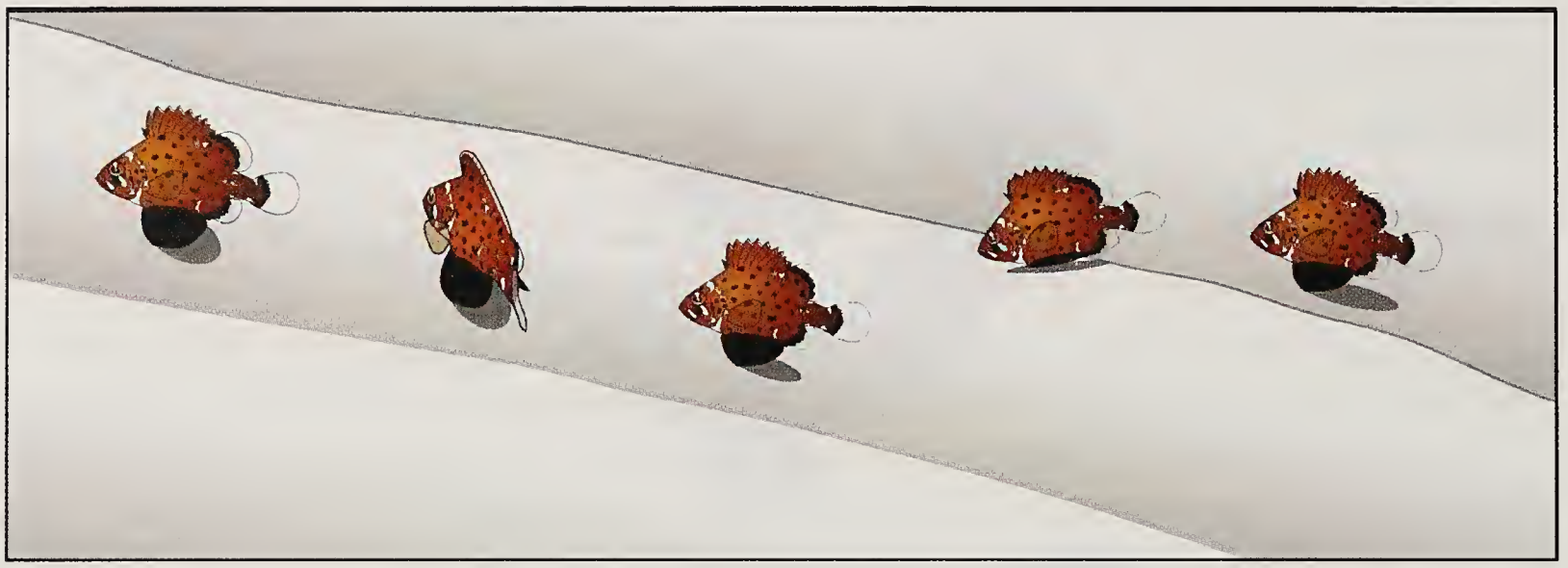

Fig. 2. Diagrammatic representation of the behaviors termed "traveling" (fish is portrayed moving right-to-left across the image). Drawn by LGA from video.

bass did not use long strands or isolated clumps of algae or artificial objects (e.g. lengths of rope or chain) for "kelping" behavior.

"Resting" behavior was similar to "kelping", because it also entailed holding position, appeared to be a predator avoidance mechanism, as well as an energy-saving device. Resting YOY giant sea bass were neutrally buoyant and found maintaining station about a centimeter or so off the bottom with dorsal and pelvic fins sometimes depressed and, at other times extended. YOY giant sea bass would "rest" a few centimeters above flat or gradually sloping bottom in areas of little to no surge. Depressions chosen by YOY giant

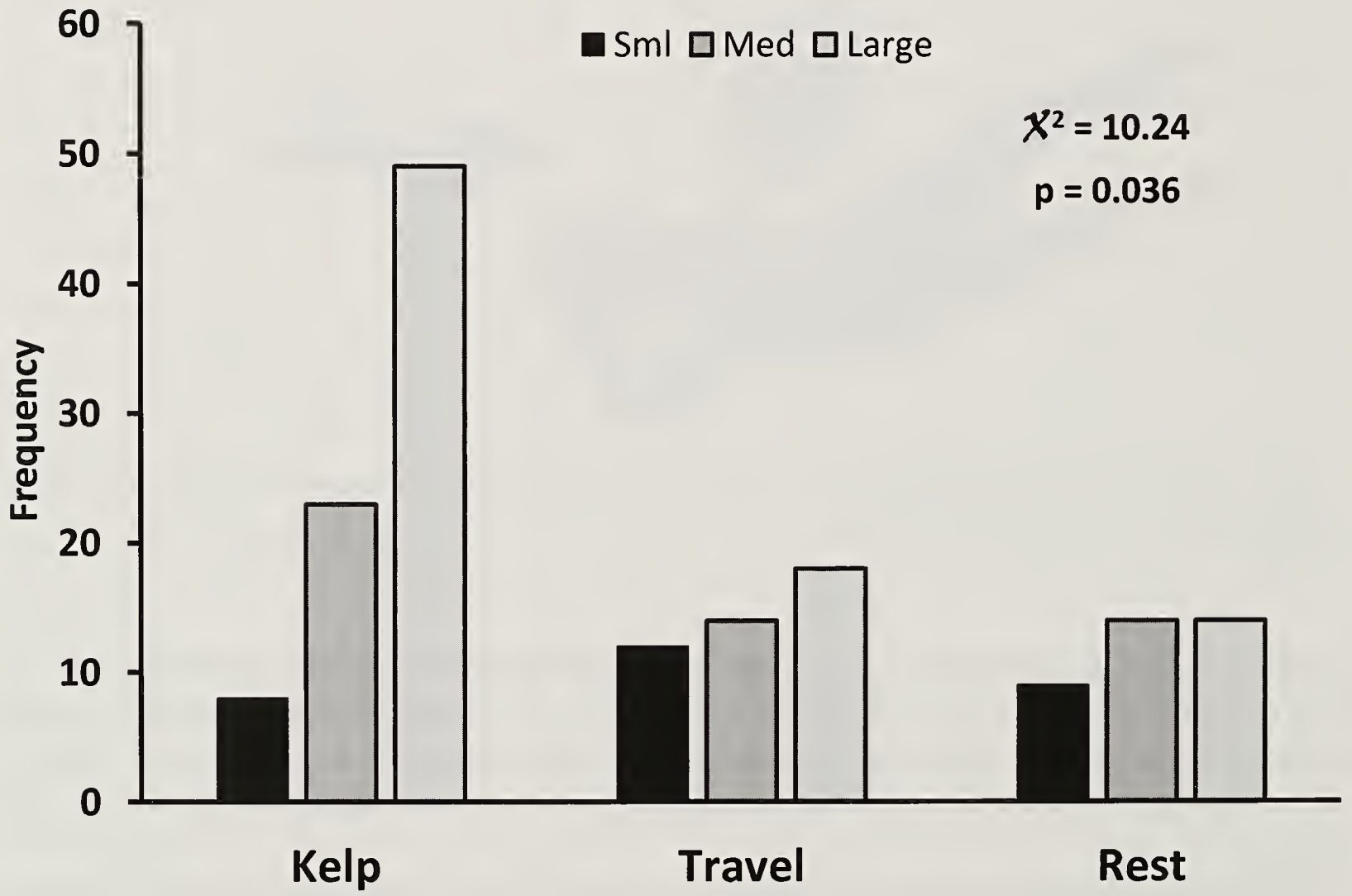

Fig. 3. The total abundances of individual young-of-the-year of *Stereolepis gigas* seen, displaying each of three behaviors at the time of sighting. Black bars represent the smaller black phases, brown bars represent medium-sized brown phases, and orange bars represent the larger orange fish.

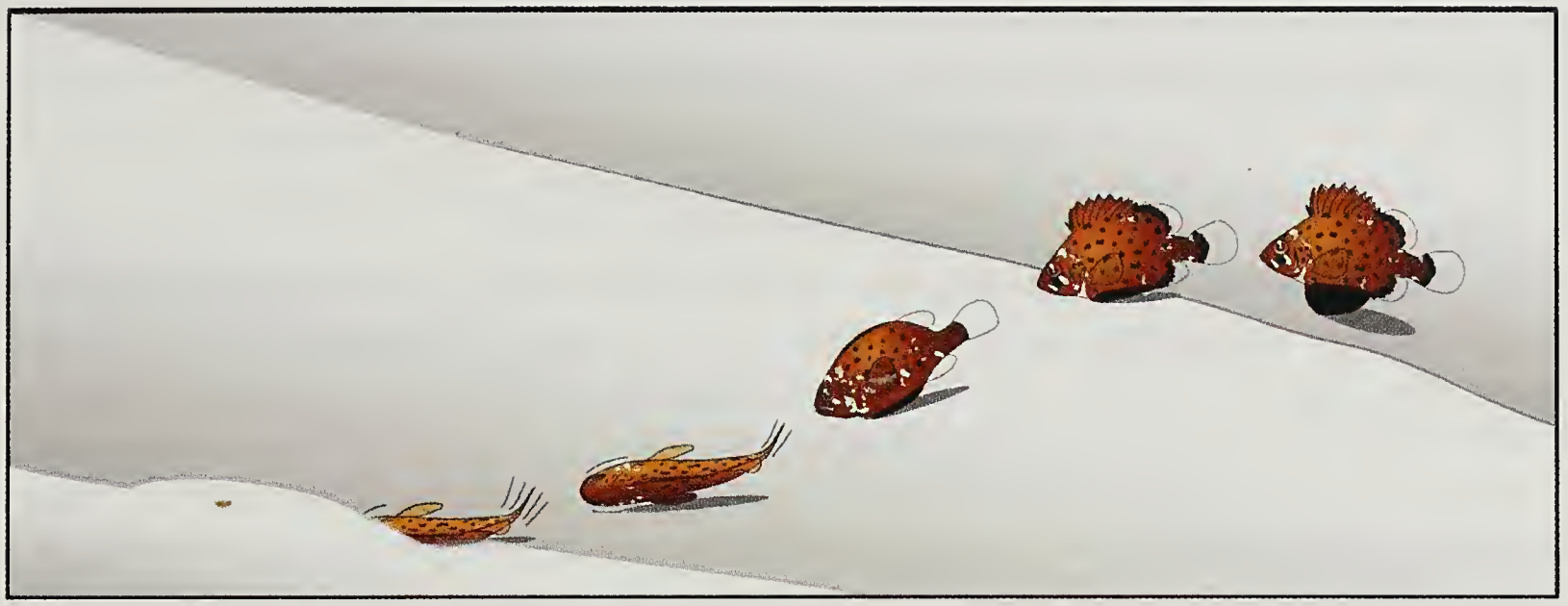

Fig. 4. Diagrammatic representation of "burying" behavior (as per Couffer and Benseman 2015).

sea bass for resting were always small, sometimes not much larger than the fish itself, but rarely larger than 30 cm across. YOY giant sea bass probably use small depressions as shelter from the near-shore surge caused by wave action, however, fish were also detected using small depressions in deeper water where there was little to no surge present. We did observe instances where fish rested in a depression even though appropriately sized detritus fragments that could have been used for kelping was scattered across the bottom. We observed fish maintaining resting postures over long periods of time. One 20 mm black-phase fish that was not disturbed by the observer hovered a centimeter or so above the bottom of the same 30 cm sand depression for over four hours during the middle of one day. That fish was surrounded by many depressions of similar depth and width. On rare occasions, an individual could be startled from its resting location and dart away quickly, but usually the fish would casually drift away as if imitating the shape, color, and movement of a detached piece of drift kelp. On occasion, resting behavior was abandoned when an observer approached. The fish would then slowly abandon the resting behavior and start to drift away, switching to the "traveling" behavior.

The behavior termed "traveling" (Fig. 3) was where an individual was observed moving a few centimeters above the bottom in a slow, steady fashion. The fish used its dorsal and large pelvic fins as steering rudders to "sail" across the bottom, extending, retracting, or angling the fins independently. The fish sometimes extended a pelvic fin and dragged it across the soft sandy substrate as it swam.

YOY giant sea bass usually drift or travel with the head angled downward, and with quick forward movements, ingesting individual mysid shrimps. Thus, they were observed to employ suction feeding, as the adults of many fishes are known to practice (Gerking 2014). Feeding behavior was observed sporadically during most observations of the previously described behaviors. The final behavior was categorized as "burying" (Fig. 4), where an individual YOY giant sea bass that was threatened by the approach of a diver completely buried itself in sandy bottom as a probable predator avoidance mechanism (Couffer and Benseman 2015). This behavior, while dramatic, was observed only on a single occasion.

On numerous occasions, we witnessed large swarms of Opossum shrimp or mysid shrimp (*Amathimysis trigibba)* within the YOY giant sea bass nursery areas. We were able to photograph and video-record these feeding behaviors many occasions. As previously reported

(Benseman and Allen 2018), we confirmed through gut content analysis that no other food item occurred more frequently in the 35 YOY giant sea bass guts examined.

Discussion

Previous studies described the larvae of giant sea bass as being heavily pigmented with both black and yellow chromatophores, having well developed fins, a rounded caudal fin, and undergoing several pigmentation phases (Shane et al. 1996), but they did not go into specific details about pigmentation. The orange, sometimes brick red-pigmented, phase described herein has been seen and photographed most often by citizen scientist divers, possibly because these fish are the most conspicuous due to their brighter color under dive lights and their larger size. Brown phase individuals are smaller than those in the orange phase are. They are less recognizable as a YOY giant sea bass and have been photographed on only a few occasions. Black phase fish are smaller still and very cryptic in appearance. Black phase individuals spent a lot of their time traveling and, at first glance, appeared as nothing more than a speck of detritus drifting in the surge. This is probably why the black phase has not been described in the wild before. That coupled with the fact that both the black and brown phases transition into the orange phase relatively quickly (Benseman and Allen 2018). Regardless of the specific color phase (black, brown, or orange) exhibited by the individual YOY giant sea bass, as our videos revealed, the attenuation of light wavelengths at depth rendered all the brown and orange phased individual YOY giant sea bass to be a typical greenish-gray color much the same as that of surrounding kelp fragments and detritus. Thus, the pigmentation of YOY giant sea bass matches their immediate surroundings and is a form of crypsis. Furthermore, the white spots on the sides of orange-phase YOY giant sea bass appear to mimic the white patches of bryozoans that are often sparsely scattered across older kelp fronds. Crypsis has been shown as a highly successful anti-predator strategy across many taxa of marine and terrestrial animals (Endler 1978, Merilaita et al. 1999).

The soft sandy benthos that surrounds these underwater canyons have been found to be successful nursery areas for giant sea bass because they are replete with small prey (Dahl 1952) and relatively devoid of predators (McLachlan 1990) especially when compared with crowded kelp forests and rocky reefs. This soft benthos offers little physical protection from visual predators yet may provide cover from predators through burying behavior. Burying is a common behavior in many teleost species, but not in quite the same manner. Senoritas, *Oxyjulis californica*, also bury themselves to avoid predation, but typically only at night and not in direct response to an approaching predator (Hobson 1965). Flatfish will also bury themselves to ambush unsuspecting prey, but usually the head and/or operculum remains unburied (Gibson & Robb 1991). However, this YOY giant sea bass completely submerged beneath the sediment and only a small section of scales could be seen (Fig. 7). This unique combination of adaptations could allow for them to hide in plain sight, but when necessary, to take refuge in the sediments and wait for the predator to abandon pursuit.

"Kelping" is probably an adaptation to the bare environment and it allows the YOY giant sea bass to hide in plain sight and avoid predation during these early developmental periods. The color of the kelp present varies from browns to oranges with scattered small white bryozoan patches, just like the coloration of YOY giant sea bass under natural underwater light. This behavior helps them to blend into the bare sandy bottom surroundings and avoid predation. In fact, they are so successful at this strategy that on numerous

occasions, divers preforming surveys overlooked fish, moving past or over the top of "kelping" fish only to be shown their mistake by the accompanying diver. Fish appeared to feed most often when traveling. The YOY giant sea bass usually travel slowly with the head angled downwards, and then with quick forward movements snap up individual mysid shrimps, after which they usually continued swimming with the current and not appearing to expend much energy. They would extend their dorsal and the enlarged pelvic fins in and out, using the surge to maneuver. The dragging of the enlarged pelvic fins against the substrate probably functioned to establish the distance between the fish and the bottom. This should prove beneficial since this habitat experiences such variable surge patterns and waves.

We believe that the individual was stabilizing itself against the surge, similar to a sailor manipulating a sail and dagger board on a sailboat. This action, coupled with the gentle dragging of the enlarged pelvic fins against the substrate while traveling (possibly to establish the distance between the fish and the bottom) could be beneficial since these areas experience such variable surge patterns and waves. Establishing distance from the bottom could prevent injury. This might explain why the pelvic fins are so greatly enlarged at this stage in their life, and are reduced once the has grown into adult form and occupies a less turbulent habitat.

Descriptions of early developmental processes and behavioral patterns are crucial for informing the life history for any species, allowing us to make increasingly intelligent decisions about fisheries management policies and future conservation efforts including the possible protection of nursery sites. In conclusion, we reiterate (as first stated by Couffer 2017, Benseman and Allen 2018, Allen et al. 2018) the importance of conserving these shallow nearshore sandy areas of the Southern California Bight, especially those opposite and adjacent to submarine canyons, as YOY nurseries that must be protected against sand replenishment and other anthropogenic disturbances of the benthos. Finally, this study is the first of its kind to provide a behavioral overview of the YOY of this internationally listed endangered species, and it bridges important gaps in our understanding of their life history. For the first time, we were able to describe the behavior and confirm the diet that are critical to the survival of the young-of-the-year of the critically endangered Giant sea bass off southern California.

Acknowledgements

This project would have not been possible without the effort and support of all our dive buddies who braved the cold to help us search for many hours for the "needle in the haystack". Mike Franklin, Richard Yan, Milton Love, and Mark Steele provided advice and guidance throughout the project. Edward DeMartini provided invaluable comments on an earlier draft of this paper. This research was supported by PADI, the International Women's Fishing Association, Nearshore Marine Fish Research Program, and the Office of Graduate Studies at CSUN. Specimens were collected by authorizations granted by Scientific Collecting Permits #13028 and #00032.

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Bull. Southern California Acad. Sci.
118(2), 2019, pp. 87–101
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Finding a Resting Place: How Environmental Conditions Influence the Habitat Selection of Resting Batoids

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Abstract.—Many batoid species will form aggregations while resting on the seafloor; however, the environmental variables that drive resting habitat selection behavior, and how it varies among species are not well understood. Bat rays (*Myliobatis californica*), shovelnose guitarfish (*Pseudobatos productus*), and round stingrays (*Urobatis halleri*) have been observed forming heterospecific aggregations. We investigated the effects of substrata type and seafloor water temperature as two likely variables that would influence resting habitat selection for these species. Spatial distribution patterns of individuals were determined via diver-based surveys over two survey seasons (Fall 2013 and Summer 2014) and related to detailed georeferenced habitat maps. While these batoids were found resting on both soft sediment types available, fine-sand was selected by all three species, whereas bat rays were the only one of the three species to select for vegetated-sand. The varying thermal sensitivities of the batoids likely influenced their responses to daily and seasonal temperatures within the study area. During Fall 2013, the three species were most abundant across a narrow temperature range (18.00 – 18.25°C); during Summer 2014, there were higher densities of bat rays in areas where daily maximum seafloor temperature reached 20°C. Each species demonstrated habitat selection decisions that were indicative of balancing tradeoffs between environmental variables. As K-selected, meso-level predators, aggregating in predictable ways can ultimately make batoids more susceptible to fishing and anthropogenic pressures. Therefore, knowledge of how batoids select their resting habitat and how environmental conditions shape distributions may provide managers with opportunities to implement better protection for resting species.

Many non-schooling elasmobranchs have been observed resting in loose aggregations on the seafloor; however, the environmental and biological variables that drive this behavior and how it varies among species are not well understood. An aggregation is defined as a group that forms when individuals seek out suitable conditions or resources, and the individuals within the group respond independently instead of using social cues or behaviors (Heupel and Simpfendorfer 2005). Individuals within the aggregations need to manage the costs (e.g., risk of parasites, competition for resources) and benefits (e.g., predator avoidance, foraging efficiency) associated with being in a group (Romey 1995; Hoare et al. 2004; Jacoby et al. 2012). Therefore, aggregations should form when the benefits outweigh the costs, leading to a potential fitness gain. However, if the costs begin to outweigh the benefits, the habitat may become unfavorable and cause individuals to move.

Elasmobranch aggregations have been observed in highly mobile species such as scalloped hammerhead sharks (*Sphyrna lewini*) and grey reef sharks (*Carcharhinus amblyrhynchos)* (Klimley et al. 1988; Economakis and Lobel 1998; Heupel and Simpfendorfer 2005),

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as well as, non-obligate ram ventilating (intermittently active), demersal species including most batoids such as cowtail stingrays (*Pastinachus sephen*) and reticulate whiprays (*Himantura uarnak*) (Semeniuk and Dill 2006). Most batoids spend a considerable amount of time resting on or in soft substrata, so it is suggested they would choose resting habitats with adequate foraging or refuging opportunities as well as suitable environmental conditions. Therefore, we hypothesized two environmental variables that influence habitat selection decisions for benthic batoid species most likely include substrata type because of their direct association with the seafloor, and water temperature because of their ectothermic physiology. Because these two key environmental variables can vary temporally (i.e., tides, seasons) and spatially (i.e., depth, geographically), some batoid species may periodically aggregate in certain areas when conditions are physiologically or behaviorally advantageous.

When batoids are observed on the seafloor, it is assumed they are resting or potentially foraging. While it can be difficult to observe foraging behaviors without influencing their behavior, observing batoids at rest can be less invasive and used to quantify how substrata type influences resting habitat selection. More susceptible batoids may use unconsolidated sediment substrata (e.g., sand, mud) (White and Potter 2004), because it offers a place for individuals to partially bury to hide from predators while at rest (i.e., refuging). At times, batoids may find suitable benthic habitat for resting, but other variables changing around them, such as temperature, could limit whether individuals choose that substratum or remain in that area.

Temperature is considered one of the key environmental variables influencing elasmobranch distributions, movements, and habitat selection due to its direct effect on physiological functions (Brett 1971; Schmidt-Nielsen 1997; Bernal and Lowe 2015). Warmer temperatures can benefit ectotherms physiologically by increasing rates of physiological processes (i.e., muscle performance, metabolism, digestion); however, if too warm, temperatures may add additional physiological costs by decreasing physiological efficiency (Economakis and Lobel 1998; Hight and Lowe 2007; Di Santo and Bennett 2011b, a). When environmental temperature changes, thermally-insensitive elasmobranchs, those with low Q_{10}s, may be able to better tolerate their chosen resting habitat. In contrast, thermally-sensitive elasmobranchs (high Q_{10}s) may be forced to move to a more thermally suitable habitat (Fangue et al. 2003; Wallman and Bennett 2006; Luongo and Lowe 2018). To compensate, some elasmobranchs behaviorally thermoregulate by shuttling between heterogeneous thermal conditions to gain an energetic advantage (Di Santo and Bennett 2011a; Bernal and Lowe 2015). For example, bat rays (*Myliobatis californica*), lemon sharks (*Negaprion brevirostris*), and Atlantic stingrays (*Dasyatis sabina*) implement a "hunt warm – rest cool" strategy where they forage in warmer waters and rest in comparatively cooler waters to decrease metabolic costs and increase digestive efficiency (Hopkins and Cech 1994; Matern et al. 2000; Di Santo and Bennett 2011b; DiGirolamo et al. 2012).

Because of their meso-predator trophic level, K-selected life history traits, and time spent resting on the seafloor, aggregating in predictable temporal and spatial patterns makes some batoid species particularly vulnerable to overfishing, (Mucientes et al. 2009; Jacoby et al. 2012), emphasizing the need to understand the drivers of habitat selection decisions for resting batoids. Big Fisherman's Cove (BFC) at Santa Catalina Island is a marine reserve with a high diversity of marine species, including high abundances of elasmobranchs. Bat rays (*Myliobatis californica*), shovelnose guitarfish (*Pseudobatos productus*) (from now on referred to as "shovelnose"), and round stingrays (*Urobatis halleri*) have all been observed aggregating in BFC, yet no studies have been done to determine what mechanisms

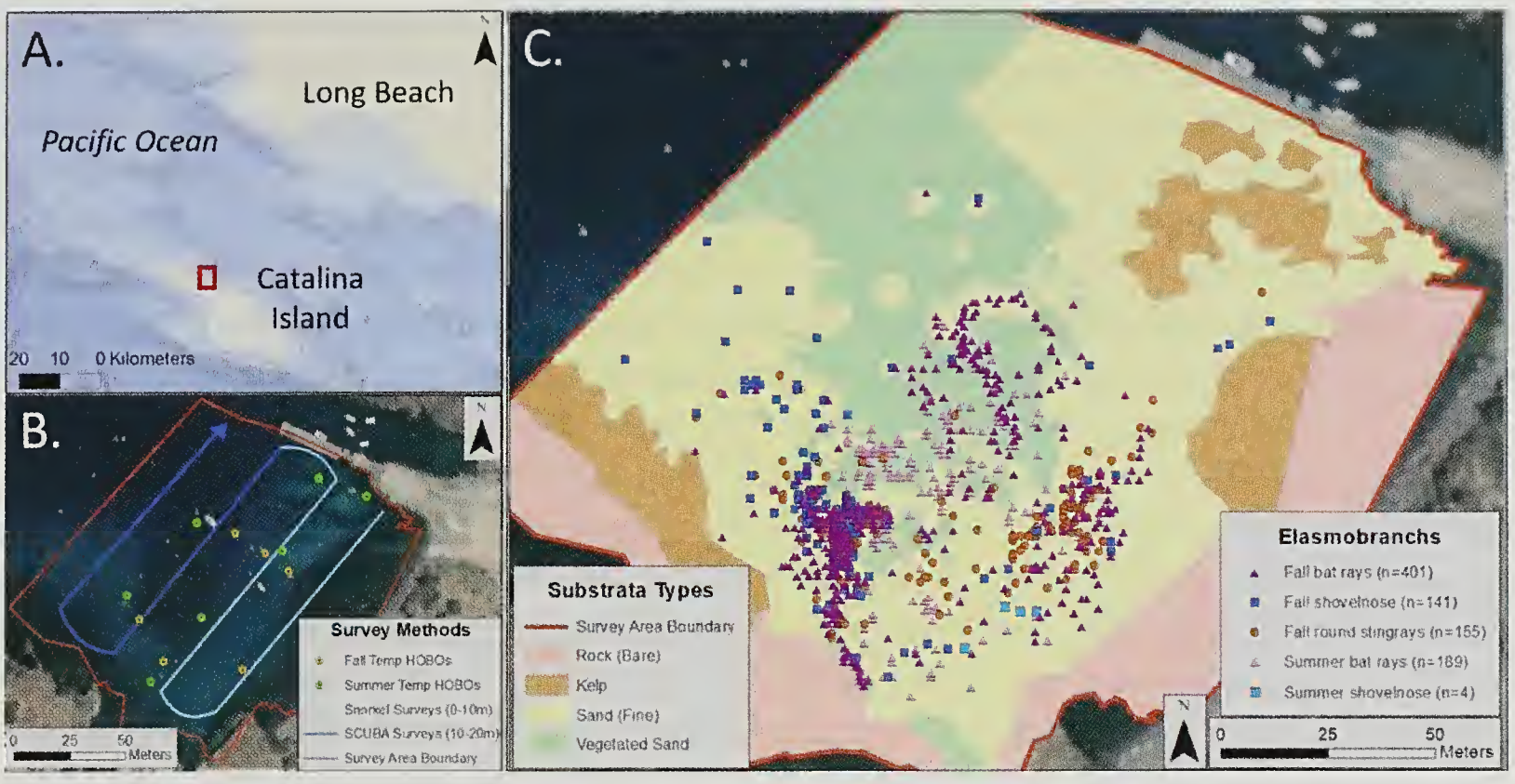

Fig. 1. A) Big Fisherman's Cove (BFC) location at Santa Catalina Island, USA. B) Survey methods for collecting batoid geolocation positions, substrata type, and locations of temperature data loggers. C) Benthic substrata map indicating different substrata classifications within BFC and positional estimates observed for each batoid during Fall 2013 and Summer 2014. Red survey area boundary (panels B and C) indicates extent of daily surveys.

drive these heterospecific aggregations. Therefore, to better understand how these three batoid species form aggregations, the goal of this study was to investigate substrata type and temperature as potential variables that drive their resting habitat selection decisions.

Materials and Methods

Diver-based visual surveys took place in Big Fisherman's Cove (BFC) at Santa Catalina Island off the southern California coast (33° 26′ N, 118° 29′ W) (Fig. 1A). Surveys occurred at different times of day in the Fall of 2013 (n = 11) and the Summer of 2014 (n = 10). Surveys were done using belt transects (width varied depending on daily visibility) that spanned the entire cove area (2.73 ha) (Fig. 1B). Transects extended approximately 165 m from the shoreline in the northwest direction, in depths no deeper than 20 m. Shallow areas (depth: 0-10 m) were surveyed from the surface via snorkeling, while the deeper areas (depth: 10-20 m) were surveyed using SCUBA. During these surveys, geo-positional fixes of all observed resting batoids and the substrata type they were resting on were recorded by a diver positioned directly over top, orthogonal to the animal, using a handheld GPS unit at the surface (Garmin GPSmap 76Cx). For the deeper areas, two divers surveyed 3 m above the seafloor while a snorkeler, connected by rope to the divers, followed from above in order to gather accurate geo-positional fixes of sighted individuals. The rope allowed divers to communicate what substrate and species to record for each positional point via a series of tugs to the snorkeler with the GPS. Geo-positional fixes were not recorded if an observed batoid was swimming to or from the area. In addition, temperature data loggers (Onset Computer Corporation: HOBOs) were distributed throughout the cove (Fall 2013, n = 6; Summer 2014, n = 7) on the seafloor to record water temperature (resolution = 0.14°C at 25°C) every 15 min (Fig. 1B).

Batoid geo-position data by species were plotted on a geo-referenced map of BFC and analyzed using ArcGIS (ArcMap vers. 10.2). To characterize the spatial areas used by the

different species, a 95% kernel utilization distribution (KUD) was calculated incorporating all positions determined for each species over the entire survey time period (all 2013 and 2014 surveys) (Worton 1987). The percent area overlap of the three species for each season was compared to take temporal differences of distribution into account. The KUDs of each species were then layered to determine the percent area overlap of the three species.

To quantify batoid substrata selection, a benthic habitat map of BFC was created by doing separate snorkeling surveys taking GPS points every 2 m. We then took those GPS points and interpolated polygons using ArcGIS to classify the habitat into hard and soft substrata (resolution approx. 5 m) (Fig. 1C). The habitat was classified into two rocky substrata categories: bare rock and rock with giant kelp (*Macrocystis pyrifera*); as well as two soft sediment substrata categories: fine-sand and vegetated-sand (with low relief macroalgae and seagrass). A habitat selection index (HSI) was used to determine each species selection for a specific substrata type. The HSI calculation was done for each species over each available substratum to create a species-specific analysis (pooling all surveys from both seasons). For each species, the HSI was calculated by dividing the proportion of positions within each substrata type by the proportion of that substrata type available in the survey area (2.73 ha). The numerator proportion was calculated by dividing the number of positions of a species within a substrata type (e.g., bat rays in fine-sand) by the total number of positions of that species in all substrata types. The denominator proportion was calculated by dividing the total area of a substrata type available (e.g., fine-sand) by the total survey area (2.73 ha). An HSI value of one or greater indicates selection, whereas a value less than one indicates non-selection of that habitat (Winter and Ross 1982). Chi-square tests in R v. 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria) were used to determine if the elasmobranchs selected substrata disproportionally from what was available in the survey area within BFC.

Temperature interpolations were done using the Inverse Distance Weighted (IDW) technique in ArcGIS (Zimmerman et al. 1999). Interpolated temperature data collected during the surveys visually showed no clumped aggregation patterns. However, Fall 2013 and Summer 2014 seasonal distributions were centered around the southwest corner of BFC where daily temperatures would reach their warmest. Therefore, to compare seasonal temperature selection and to determine if the warmest daily temperatures were driving distributions of batoids in BFC, we used daily (9:00 – 17:00 hrs) maximum seafloor water temperatures. To compare batoid abundance with daily maximum temperatures, the maximum interpolated temperature of each individual's location was recorded and grouped into corresponding 0.25°C temperature bins. To quantify disproportional temperature use, a fishnet grid was created with ArcGIS that consisted of 5 m^2 cells. Mean maximum sea floor water temperature and number of individuals of each species were extracted from each cell. Cells of all surveys were pooled together to construct histograms for each batoid in R to determine how each species selected temperature compared to what was available in the survey area. Chi-square tests were used to determine if the elasmobranchs significantly selected temperatures disproportionally from what was available in the cove.

Mean maximum sea floor water temperature, major substratum type (> 50%), and the total number of individuals of each batoid were extracted from each grid cell. Hurdle models were then conducted in R to statistically quantify how the environmental variables predicted abundance of each species. The hurdle model is a two part model; 1) a binomial probability model that determines presence or absence of individuals of a species in a grid cell, then 2) a truncated count data distribution, which describes the positive outcomes, or presence of individuals, based on substrata type and temperature (Cameron and Trivedi

2013). To determine what variables affected presence of each species within a grid cell, a logistic regression was run with a binomial distribution of either presence or absence of individuals within a cell. Once the first "hurdle" was crossed, the second step of the hurdle model used general linear models (GLMs) to identify which environmental variable best described the abundance of individuals. Only substrata types that were selected by the elasmobranchs were included in the GLMs to improve our ability to determine a difference in elasmobranch abundance among selected substrata types. An additional GLM was run with bat ray positions from Fall 2013 and Summer 2014 pooled together to compare across seasons.

Results

The daily mean (± SD) abundances of batoids counted per survey during Fall 2013 was 38 ± 29 bat rays, 18 ± 10 shovelnose, and 26 ± 16 round stingrays. The mean (± SD) daily abundances counted during Summer 2014 was 19 ± 13 bat rays, and 1 ± 1 shovelnose. During Fall 2013, the resting distribution of all three batoids shared an overlapping area of 0.045 ha, only 1.6% of the total survey area (2.73 ha). During Summer 2014, the resting distribution of bat rays and shovelnose had an overlapping area of 5×10^{-5} ha (only 2 individuals overlapped throughout the summer). Due to the absence of round stingrays and low abundance of shovelnose during Summer 2014, positional data for both seasons were pooled for KUD analysis. The resulting resting distribution area of the three batoids from all surveys encompassed a total area of 1.02 ha, 37% of the total surveyed area (2.73 ha). However, the overlapping distribution area of all three batoids encompassed 0.087 ha, comprising approximately only 3% of the surveyed area of BFC (2.73 ha), indicating some segregation by species groups (Fig. 2).

Out of the four substrata types available in BFC, batoids were only found resting in the two soft substrata: fine-sand and vegetated-sand. Bat rays ($X^2 = 288.76$, df = 3, $p < 0.001$), shovelnose ($X^2 = 132.76$, df = 3, $p < 0.001$), and round stingrays ($X^2 = 147.47$, df = 3, $p < 0.001$) all selected their substrata disproportionally from what was available in BFC. All three species were found to select fine-sand substrata, while only bat rays showed additional selection of vegetated-sand (HSI values: bat rays: fine-sand = 1.59, vegetated-sand = 1.19; shovelnose: fine-sand = 2.01, vegetated-sand = 0.25; round stingrays: fine-sand = 2.03, vegetated-sand = 0.21) (Fig. 3).

Daily temperature fluctuations within the study area never exceeded more than 3°C. The highest seafloor temperatures for Fall 2013 and Summer 2014 were 19.25°C and 20.75°C, respectively. During Fall 2013, the highest abundance of positions for all three batoids was between 18.00-18.25°C (Chi-square test: $X^2 = 190.3$, df = 25, $p < 0.001$) (Fig. 4). Bat rays displayed a random distribution during Fall 2013 where not all individuals were observed resting in the warmest waters; however, when daily maximum temperatures reached 20°C in Summer 2014, bat rays demonstrated a clumped distribution pattern (Fig. 5). All three batoids used water temperatures disproportionally to what was available (bat rays: $X^2 = 174.96$, $p < 0.001$; shovelnose: $X^2 = 60.6$, $p < 0.001$; round stingrays: $X^2 = 51.87$, $p < 0.001$) (Fig. 6).

Results of the hurdle model showed that in Fall 2013, temperature predicted the presence of bat rays ($p = 0.001$), but substrata type did not ($p = 0.09$). While temperature determined presence, neither substrata ($p = 0.49$) nor temperature ($p = 0.35$) predicted bat ray abundance. In Summer 2014, both temperature ($p < 0.001$) and substrata type ($p < 0.001$) predicted the likelihood of bat ray presence, while neither substrata type

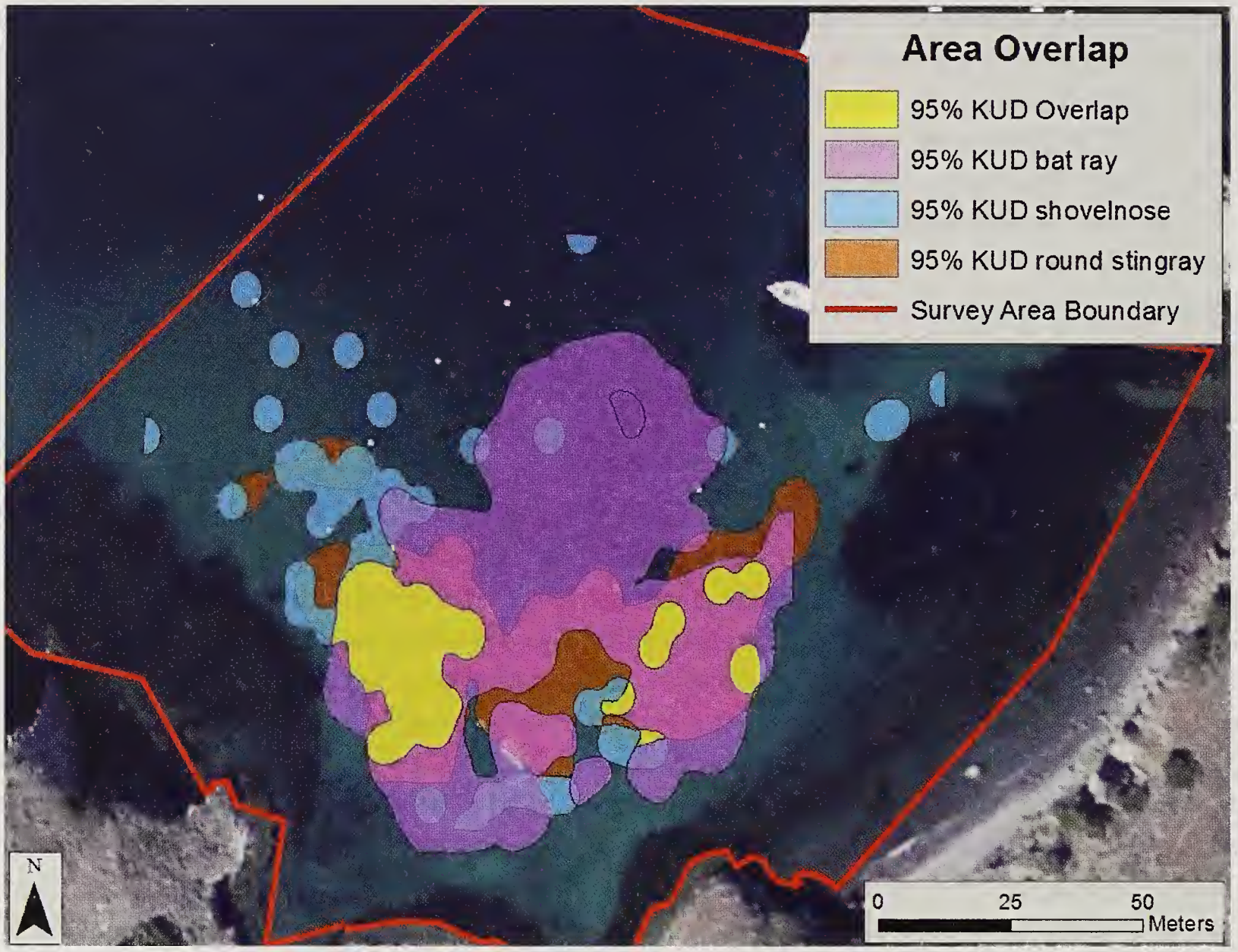

Fig. 2. Map of the 95% kernel utilization distribution (KUD) areas for bat rays, shovelnose, and round stingrays for all positional data from both Fall 2013 and Summer 2014. The yellow area indicates the overlap of KUD areas for all three species for both survey seasons (37% of total area used by the batoids).

($p = 0.07$) nor temperature ($p = 0.93$) predicted abundance. However, when all positions for bat rays from both seasons are pooled together, temperature predicted both presence ($p < 0.001$) and abundance of rays ($p = 0.04$), while substrata type did not (presence: $p < 0.11$; abundance: $p = 0.75$) (Fig. 7A). For shovelnose, substrata type predicted both presence ($p < 0.001$) and abundance ($p < 0.001$), while temperature did not predict either presence ($p = 0.16$) or abundance ($p = 0.97$) (Fig. 7B). Round stingray presence was influenced by both temperature ($p < 0.001$) and substrata type ($p < 0.001$), while neither substrata type ($p = 0.42$) nor temperature ($p = 0.34$) influenced abundance (Fig. 7C).

Discussion

Our study assumed the batoid species lying on the seafloor had chosen suitable habitat for them to rest or take refuge. Smaller species or individuals could potentially be more at risk causing them to take refuge by burying within the substrata more often than larger individuals. Fine-sand substrata likely provides more suitable burying habitat for batoids, whereas vegetated-sand substrata alternatively provides low-relief vegetation for crypsis, and potentially higher benthic in- and epi- faunal densities (Thrush et al. 1991; Cross and Curran 2000). While all three species were found resting on both soft substrata types available (fine-sand and vegetated-sand), fine-sand was selected by all three species, whereas bat rays were the only one of the three species to additionally select for vegetated-sand. Round stingrays and shovelnose are light brown to gray in color and can blend in while

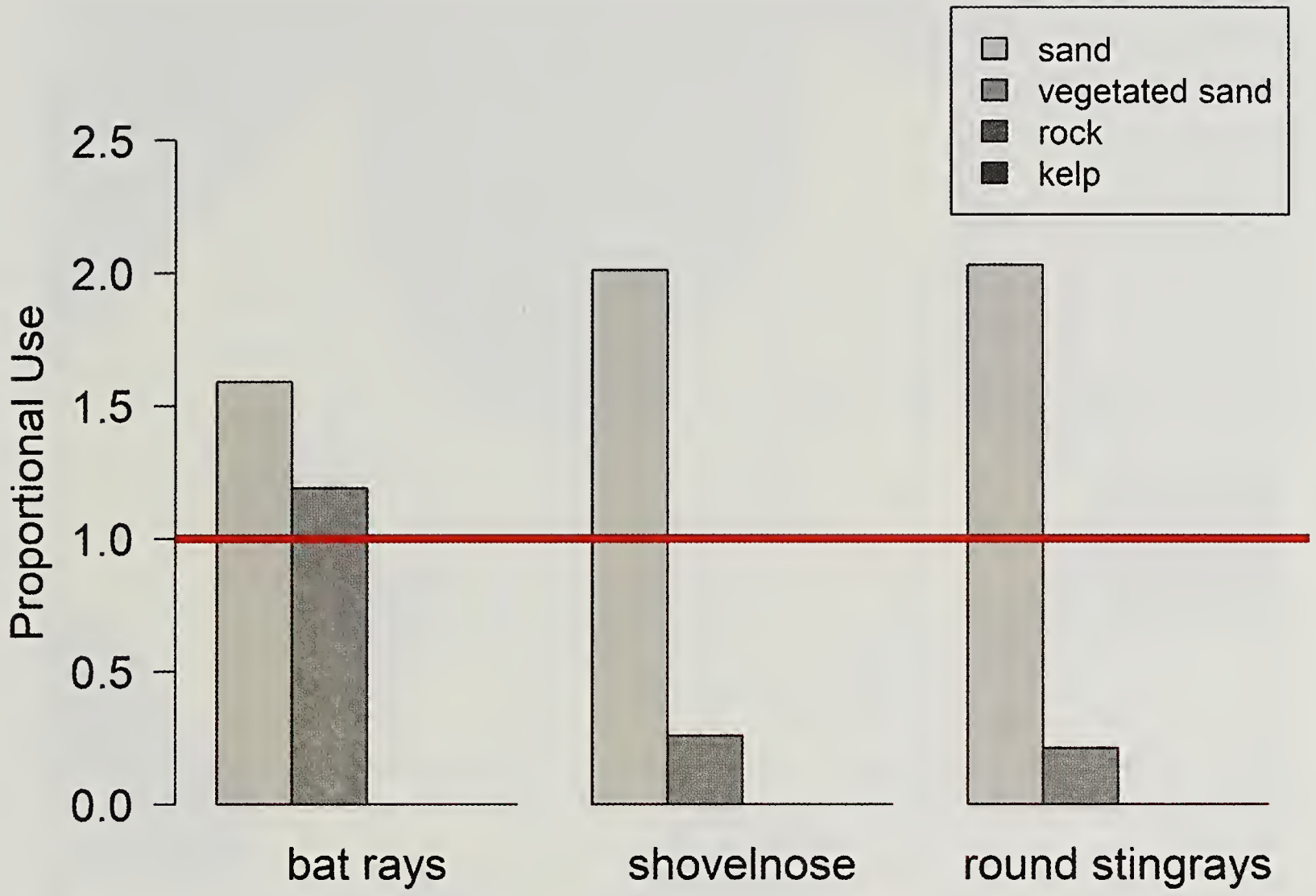

Fig. 3. Habitat selection index (HSI) for substrata types for the three batoid species surveyed in both Fall 2013 and Summer 2014. The red horizontal line designates the HSI selection value of 1.

partially buried or resting on fine-sand substrata. Bat rays on the other hand tend to be darker on their dorsal surface, which could explain why juveniles were observed buried in shallow, fine-sand areas, while larger bat rays were observed resting on the vegetated-sand substrata while at rest.

Within species, body size may have influenced substrata and temperature selection. While size of each resting individual was not recorded, estimated age classes including juvenile bat rays, juvenile shovelnose, and all round stingrays were typically found in shallower, warmer, fine-sand substrata, while adult shovelnose and adult bat rays were found in relatively deeper, cooler, vegetated-sand substrata. Previous studies have demonstrated ontogenetic shifts in thermal preference where larger individuals tend to prefer cooler water temperatures (Magnuson et al. 1979; Hopkins and Cech 2003), which may explain the size stratified distributions we observed among the three species.

Because of varying body sizes, these species likely differ in their thermal sensitivities; however, it is unclear how daily temperature ranges influenced fine-scale temperature selection. There was no overlap between Fall 2013 (17.50 – 19.25°C) and Summer 2014 (19.50 – 20.50°C) temperatures, and daily ranges never exceeded more than 3°C. Interestingly, all three species were most abundant across a narrow temperature range (18.00 – 18.25°C) during Fall 2013. This suggests that daily temperature ranges may have provided a thermal refuge by allowing these species to rest for longer periods of time, potentially allowing a physiological advantage (Bernal and Lowe 2015). However, based on the variation in daily abundances and distributions, we hypothesize that all three species periodically leave BFC (i.e., for foraging, other preferred environmental conditions); however, the temporal and spatial scale of these patterns (i.e., diel, seasonal) may vary by species.

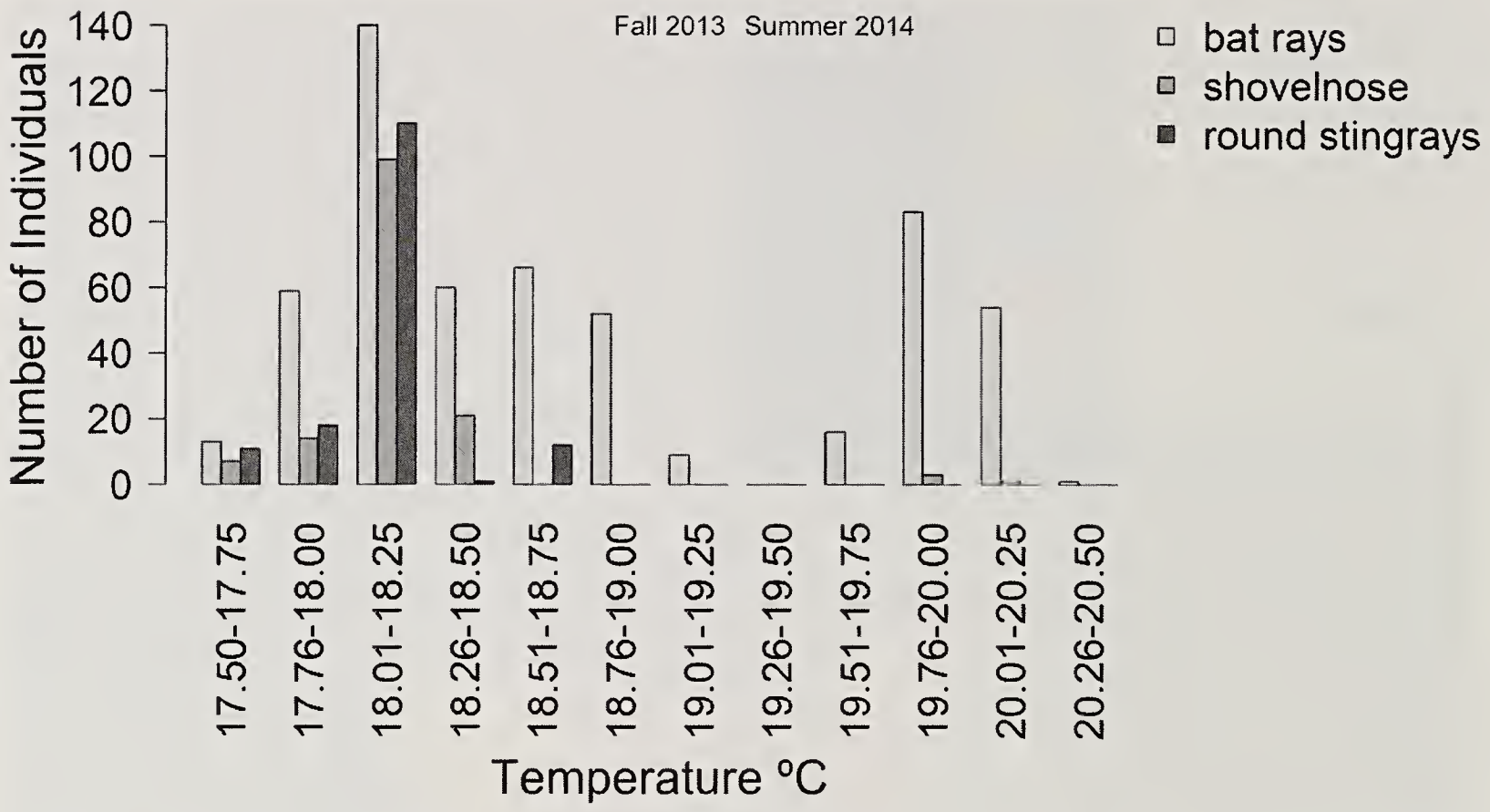

Fig. 4. Pooled extracted temperature data from the daily maximum inverse distance weighted interpolations (IDWs) for all species from all surveys. Summer 2014 temperature ranges are shaded in gray.

While this study was done during warmer seasons (e.g., Summer and Fall), colder seasons (e.g., Winter and Spring) might result in different temperature selection and distribution patterns. For instance, during Summer 2014, there were higher densities of bat rays in areas where daily maximum seafloor water temperatures reached 20°C. This seasonal peak in temperature may act as a threshold for adult bat rays where there is an increased benefit to metabolic performance or reproduction (i.e., shortening gestation) (Wallman and Bennett 2006; Jirik and Lowe 2012). Martin and Cailliet (1988) found in Elkhorn Slough, California, ovulating females were only present during summer months. Therefore, the observed summer bat ray aggregations could have been mostly females seeking a reproductive advantage, similar to leopard sharks also found in BFC (Hight and Lowe 2007). We could not easily determine the sex of resting individuals during surveys for this study, so the ratio of females to males in these summer aggregations in BFC is unknown.

Thermal preferences and sensitivities among the three batoid species may explain some of the heterospecific variation in daily abundances and spatial distributions. Bat rays can be extremely sensitive to temperatures outside 14-20°C and have a high unacclimated metabolic Q_{10} of 6.81 (Hopkins and Cech 1994). During daytime observations, bat rays in BFC may have been "shuttling" between habitats (i.e., warmer, fine-sand vs. colder, vegetated-sand) to optimize net energetic gain according to the "hunt warm – rest cool" hypothesis (Matern et al. 2000; Bernal and Lowe 2015). Round stingrays, in contrast, are the smallest of the three batoid species, implying they may have the lowest thermal tolerance, though their thermal sensitivity or metabolic Q_{10}s have not yet been quantified (Hoisington and Lowe 2005). Seal Beach, California is a location where high densities of round stingrays seasonally aggregate because of the warm water effluent from the San Gabriel River (Babel 1967; Hoisington and Lowe 2005; Vaudo and Lowe 2006; Jirik and Lowe 2012). While stingrays are present throughout the year at Seal Beach, abundance varies with season and residence time is only approximately two weeks (Hoisington and Lowe 2005; Vaudo and Lowe 2006). Because of these patterns, it has been proposed that

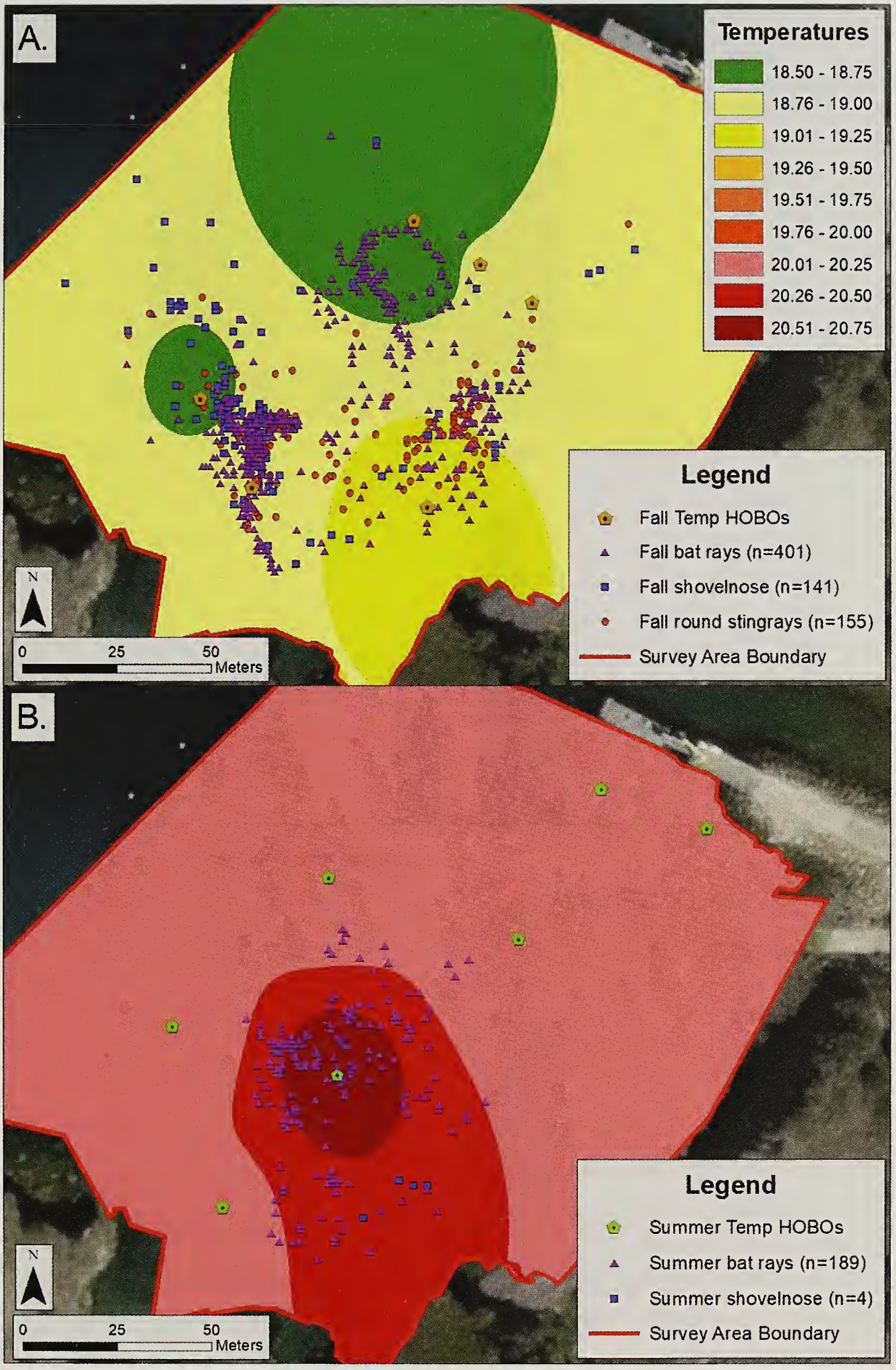

Fig. 5. Temperature IDW interpolations for the average maximum temperatures for both Fall 2013 (A) and Summer 2014 (B) survey seasons. There was no overlap between Fall 2013 (17.50 – 19.25°C) and Summer 2014 (19.50 – 20.50°C) temperatures. Positional estimates observed for each species are presented for each respected season, as well as locations of temperature data loggers for each season.

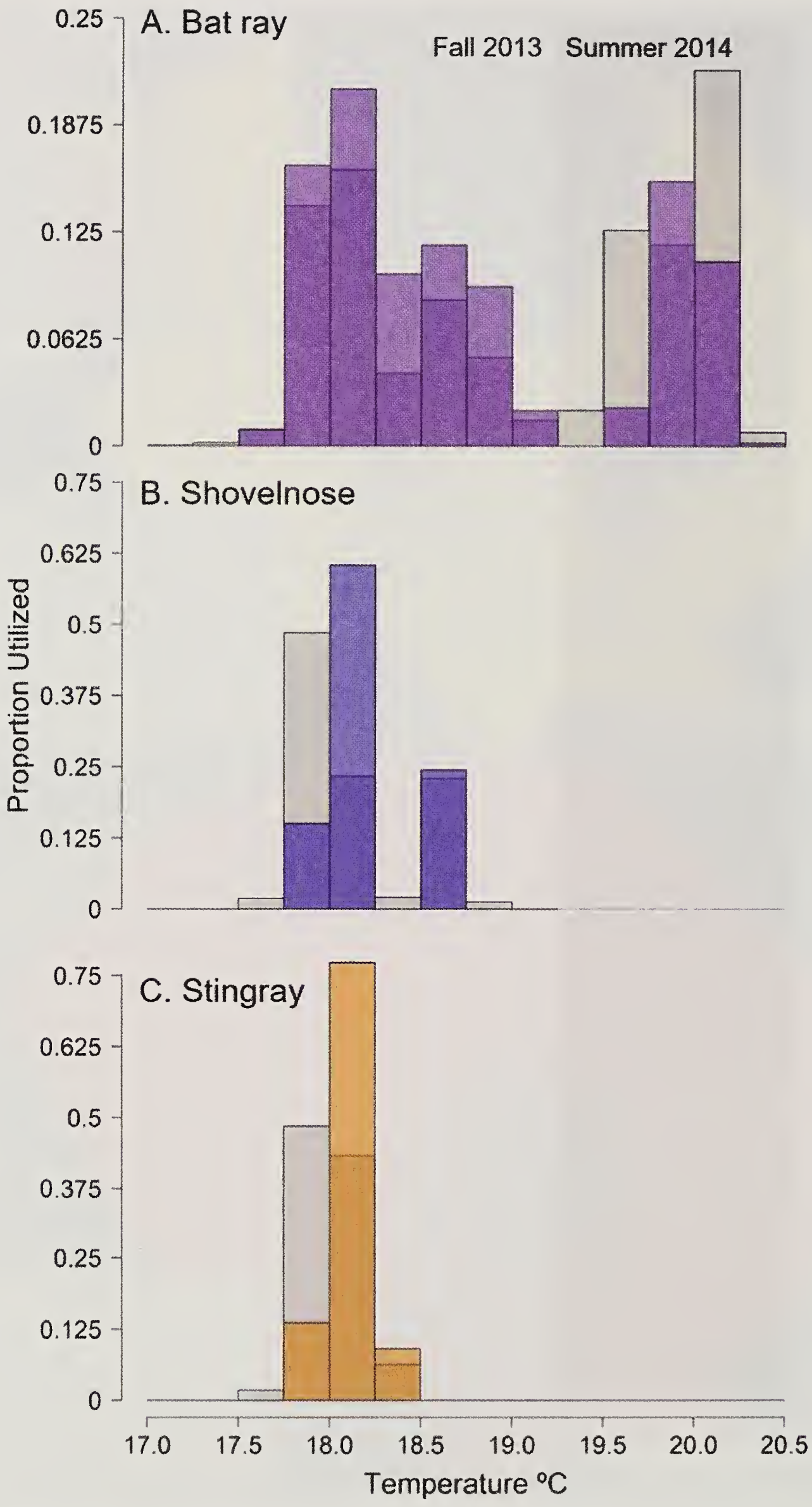

Fig. 6. Disproportional temperature use for all three batoid species for both seasons (shaded region shows Summer 2014). Gray bars represent temperatures available, whereas colored bars represent the proportions of temperatures used by individuals.

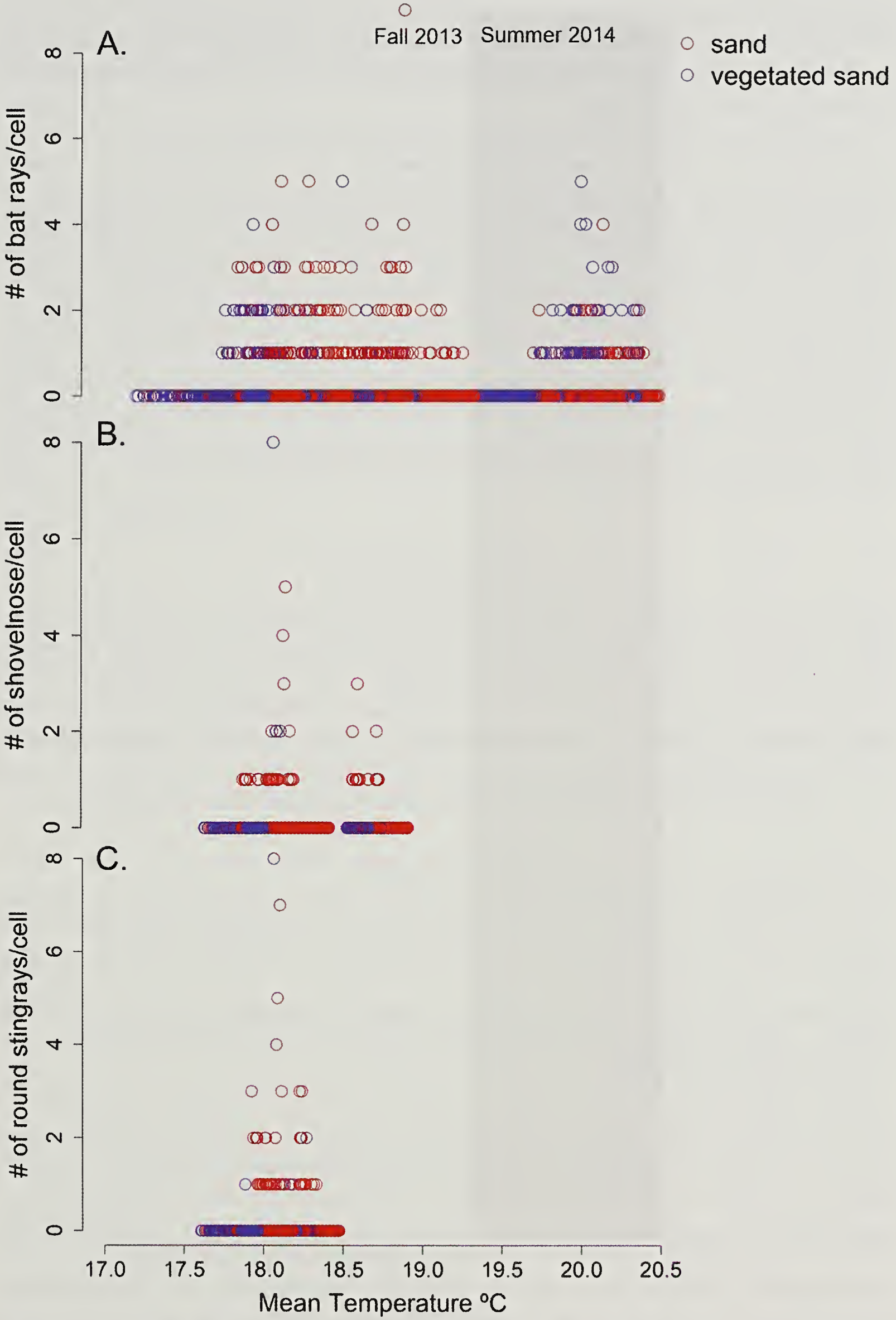

Fig. 7. General linear model results for bat rays (A), shovelnose (B), and round stingrays (C) for both Fall 2013 and Summer 2014. Substrata type is indicated by color, red denotes fine-sand and blue denotes vegetated-sand.

these Seal Beach aggregations may serve some seasonal reproductive benefit (Mull et al. 2008; Jirik and Lowe 2012). Likewise, the round stingrays aggregating in the present study may also be seeking a reproductive advantage by seeking out warm waters with preferred substrata for refuge while at rest.

Surprisingly, temperature did not influence the presence and distribution of shovelnose, whose thermal sensitivity and metabolic Q_{10}s have also not yet been quantified. Farrugia et al. (2011) conducted a comprehensive study of shovelnose movements and habitat use in the Bolsa Chica Full Tidal Basin (BCFTB) and found shovelnose prefer temperatures around 22°C. In contrast to BFC, the BCFTB is a shallow estuarine environment that has greater daily changes (12 – 30°C) in temperature compared to BFC (Espinoza et al. 2011; Freedman et al. 2015; Freedman et al. 2017). Since 22°C was rarely observed in BFC, one might expect shovelnose to instead select for the warmest possible temperatures; however, that was not the case. There is growing evidence that shovelnose may be more migratory than previously thought; therefore, it is possible they are less responsive to temperature than the other two species, and instead prioritize other environmental variables, or social aspects of aggregations, that we did not measure in this study (Espinoza et al. 2011; Nosal et al. 2014; Freedman et al. 2017).

Our Summer 2014 survey season was the beginning of the 2014 – 2016 El Niño Southern Oscillation (ENSO) event, which are known to affect distribution patterns and geographical ranges of many temperate fishes. This could potentially explain why there was no overlap in seasonal temperatures, and why abundances and distributions of the three species differed between the two warm seasons (e.g., Fall and Summer). For instance, shovelnose had the lowest mean daily abundances in Fall 2013, and only a few occurrences during Summer 2014. Farrugia et al. (2011) found shovelnose to be non-philopatric which would explain the high variability of shovelnose presence during our study. Additionally, there were no round stingrays present in Summer 2014, possibly due to reduced prey populations, or because ENSO conditions provided suitable resting conditions elsewhere.

Our study investigated what we hypothesized to be the two most important environmental variables that would drive heterospecific aggregations. While substrata type and temperature did influence batoid habitat selection decisions, other environmental variables such as depth, tidal height, salinity, and dissolved oxygen could additionally effect where these species choose to rest. For example, because batoids are dorso-ventrally flattened, they may be able to use tidal flows for transportation (Teaf 1980; Smith and Merriner 1985). Round stingrays have been shown to exhibit movement patterns consistent with daily tides (Vaudo and Lowe 2006), yet bat rays demonstrated movements correlated with temperatures but not tides (Matern et al. 2000).

Additionally, while our study did not measure any social interactions or behaviors among the three species, we are not discounting sociality as one of the potential reasons for batoid aggregations. Semeniuk and Dill (2006) found instead of relying on others as evidence of suitable resting conditions, batoids may actively seek out resting partners for other benefits that aggregating can provide. For instance, smaller, more susceptible individuals (e.g., round stingrays, juvenile bat rays) may aggregate to increase vigilance and to flee in groups when necessary, as opposed to individually. It is possible that the heterospecific aggregations we observed are formed because more susceptible species can take advantage of greater sensory capabilities of the other species (Semeniuk and Dill 2006). In addition, since these batoid species are benthic foragers, they may use social cues (both con- and heterospecific) to help find prey along the seafloor. While we assumed the individuals recorded for this study were at rest, these batoids could have also been waiting to detect prey along

the seafloor (e.g., water jets from siphons) (Matern et al. 2000). Understanding the relationship between foraging and habitat selection of batoid species is ecologically important since their foraging behavior can result in high sediment turnover and altered invertebrate communities.

Conclusions

When faced with choosing a resting place, bat rays, shovelnose, and round stingrays must balance tradeoffs between substrata type (i.e., soft sediment for refuge and potentially prey-rich environments for foraging) and temperature (i.e., physiological advantages). It is important to understand how meso-level predators such as batoids respond to these variables to make predictions as to how changing ocean temperatures and coastal urbanization may affect the diel and seasonal distribution patterns and behaviors of these batoid species. Resource managers can use this information to better protect these aggregating, K-selected species in their preferred habitats. Moving forward, research should focus on environmental and social aggregation mechanisms of batoids to create the most effective approach for understanding habitat selection decisions and implementing proper protection and restoration plans.

Acknowledgements

We thank the California State University Ocean Studies Institute and USC's Wrigley Institute of Environmental Studies for the opportunity to conduct this work in Big Fisherman's Cove, Santa Catalina Island. Thanks to J. Ohde and B. Stirling for fieldwork assistance, as well as B. Ahr, M. Schram, C. White, and D. Crear for assistance with spatial and statistical analyses. We thank the three anonymous reviewers who significantly improved the quality of this manuscript. This research was done as part of the CSU Catalina Marine Biology Semester with funding provided by the SCTC Marine Biology Foundation.

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Bull. Southern California Acad. Sci.
118(2), 2019, pp. 102–108
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Description of a Male Urogenital Papilla in the California Grunion, *Leuresthes tenuis*, a Beach-spawning Marine Silverside Fish

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The California grunion, *Leuresthes tenuis* (Ayres, 1860), like its sister species, the Gulf grunion, *L. sardina* (Jenkins & Evermann, 1889), engages in unusual reproductive behavior in which males and females aggregate on sandy beaches to spawn (Thompson 1919; Walker 1949, 1952; Thomson and Muench 1976; Martin 2015). Spawning by California grunion occurs at night during semilunar spring high tides in March-August, from Magdalena Bay, Baja California, Mexico, to Tomales Bay, California (Walker 1952; Moffatt and Thomson 1975; Roberts et al. 2007; Martin et al. 2013). Females deposit eggs ~5-10 cm deep in the sand while males release sperm as they surround females at the sand surface. The externally fertilized eggs develop within the sand until competent to hatch. Hatching is triggered when the embryos are washed out of the sand during a subsequent spring high tide series, and the swimming larvae are washed into the ocean (Thompson 1919; Griem and Martin 2000; Martin et al. 2011). While extracting gametes for other studies, we noticed a structure associated with gamete release in California grunion males, but not females. In this study, we used dissections, magnetic resonance imaging (MRI), paraffin histology and light microscopy to characterize that structure as a muscular urogenital papilla, a sexually dimorphic character that can be used to distinguish the sexes noninvasively. We also propose a function for the urogenital papilla in external fertilization in this beach-spawning species.

Adult California grunion were collected during spawning runs on Cabrillo Beach, San Pedro, CA (GPS coordinates: 33°42′33″N, 118°16′59″W), and Doheny State Beach, Dana Point, CA (33°27′43″N, 117°41′14″W), under California Department of Fish and Wildlife Scientific Collecting Permits (SC-3211, SC-4783, SC-10567, and SC-10585). Whole fish were placed on ice or dry ice, or euthanized in tricaine methanesulfonate (MS-222; 0.2 g L^{-1} of seawater) and fixed in 10% phosphate-buffered formalin, and then transported to California State University Fullerton (CSUF). Iced or frozen and thawed whole males (N = 6) were dissected to examine the urogenital papilla and its relationship with other organs, and several females were also dissected to examine the ovary and oviduct. One fixed male and one female were prepared for MRI, and segments of other fixed individuals (15 males and 8 females) were processd for histology. All work was approved by the CSUF Institutional Animal Care and Use Committee under protocols 08-R-07, 11-R-07, 14-R-07, and 17-R-06.

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The MRI image data were acquired at the Keck Center for Functional MRI at the University of California San Diego on a 7T (300 MHz, 20 cm bore) small animal imaging system (Bruker Biospec Avance II, Bruker AXS Inc., Madison, WI) fitted with a 72-mm inner diameter quadrature RF volume coil (Bruker Biospin GmbH, Ettlingen, Germany). All images were acquired with a standard T1-weighted 3D fast spoiled gradient recalled echo acquisition pulse sequence with images collected in the transverse plane. The entire body of a male specimen was scanned at an isotropic voxel resolution of 100 μm using the following pulse sequence parameters: 15°FA, 26.6 ms TR, 13.2 ms TE, 300 kHz bandwidth, and 3 averages. An additional higher resolution scan of the midbody region containing the urogenital papilla was then acquired at 80 μm isotropic resolution (with 6 averages). The corresponding midbody region of a female was also scanned at 80 μm using the same pulse sequence parameters as the male. The MRI data are freely available upon request in the original 32-bit integer format or other standard medical imaging formats at the Digital Fish Library (http://www.digitalfishlibrary.org/library/ViewSpecies.php?id=334). MRI image data of the male in NIfTI format were segmented using ITKSnap (ver. 3.6.0, http://www.itksnap.org/pmwiki/pmwiki.php) and smoothed and rendered in 3D using Blender (ver. 2.79b, https://www.blender.org), to reconstruct the wall of the urogenital papilla, the distal intestine to the anus, the sperm duct from the two testes to the genital pore, and the mesonephric urinary duct from the trunk kidney to the urinary pore.

The histology samples were rinsed in phosphate buffer, dehydrated in a graded ethanol series, cleared with xylenes, and infiltrated and embedded in paraffin wax. Tissue blocks were sectioned with a rotary microtome and 5-μm-thick sections were mounted on glass slides and stained either with hematoxlyn and eosin or with Mallory's trichrome (Humason 1979). Transverse sections and sagittal sections of males containing the urogenital papilla, and of females in the corresponding body regions, were examined. Sections of isolated papillae dissected from formalin-fixed males were also prepared and examined.

Normally, in live male grunion collected while spawning on shore, the urogenital papilla does not protrude from the body. When pressure was applied externally to the ventral side of a male grunion, the papilla would project externally (Fig. 1A) and sperm could be ejected a considerable distance (more than one body length). Applying pressure to the ventral surface of female grunion released unfertilized eggs but did not result in protrusion of a corresponding structure. A lateral view of a male grunion that was partially dissected (Fig. 1B) shows the urogenital papilla to be a vascularized, muscular structure between the anus and anal fin. In males that were examined under a dissecting microscope, application of pressure caused sperm to flow from the sperm duct within the papilla and fecal material to exit via a separate anal opening. Injections of ink and MRI imaging confirmed that the sperm duct within the urogenital papilla connects with the testes. The MRI and histological analyses revealed additional details about the structure of the papilla, indicating that it contains both the sperm duct and the mesonephric urinary duct, and how it differs from the oviduct of the female. MRI images of longitudinal sections of the male (Fig. 1C, D) show the muscular wall of the urogenital papilla and its position relative to the intestine, testes, and urinary duct. The 3D segmentation of the MRI data (Fig. 1E) traces the distal intestine to the anus, the posterior region of the testes to the sperm duct, and the urinary duct descending from the posterior region of the trunk kidney into the urogenital papilla in a position posterior and dorsal to the sperm duct. Transverse sections of males provide more details of the papilla's structure and the relative positions of the sperm duct, urinary duct, distal intestine, and anus (Fig. 2). In more anterior sections (Fig. 2A, D, E), the

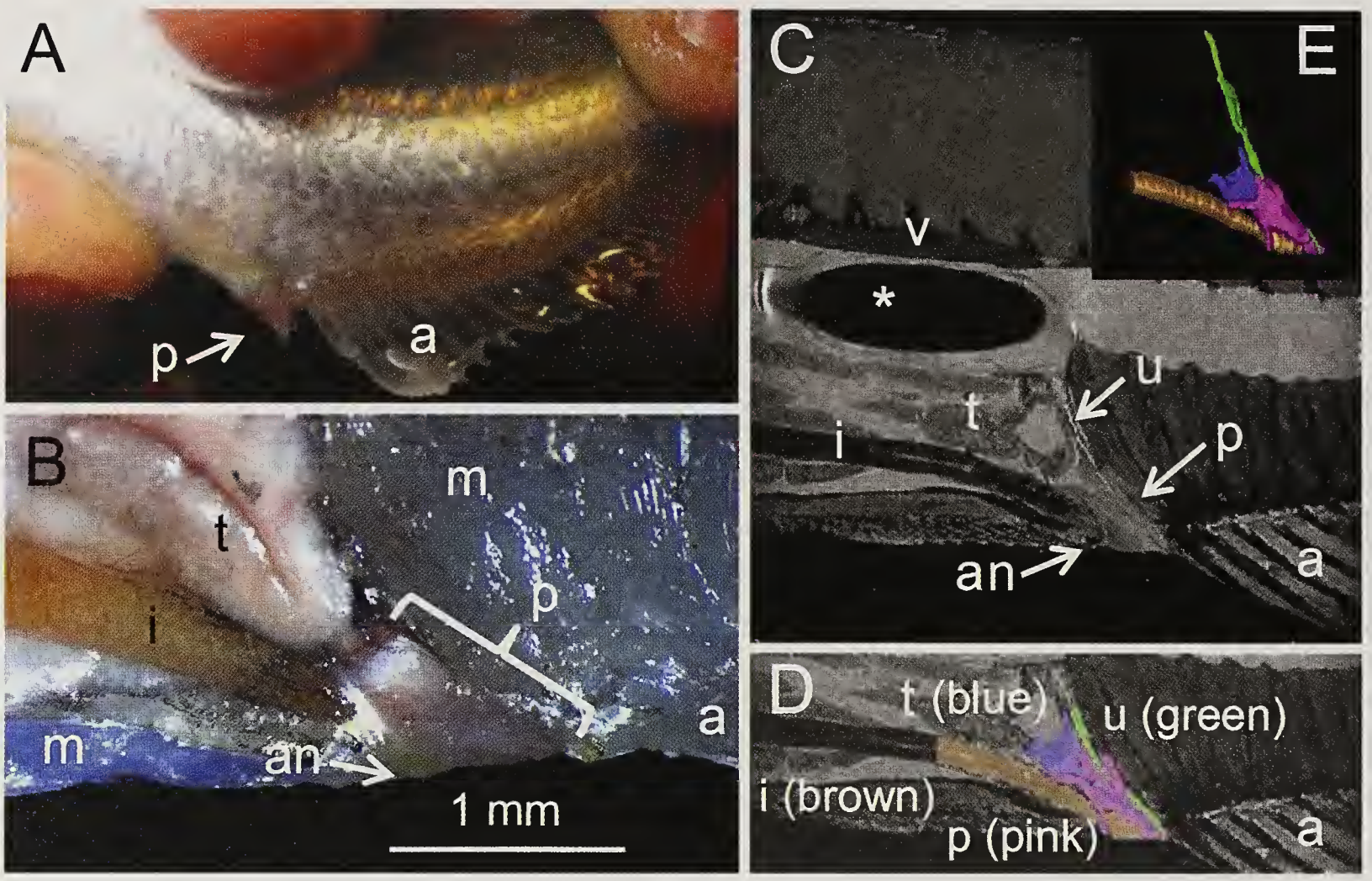

Fig. 1. Urogenital papilla in male California grunion, *Leuresthes tenuis*. A. Lateral view of the midbody region of a male with urogenital papilla (p) protruding just anterior to the anal fin (a). Anterior of fish is to the left. B. Lateral view of partially dissected male grunion showing vascularized, muscular urogenital papilla (p), located between the intestine (i) and anus (an) and the anal fin (a), and through which sperm is released from the testes (t). m = body wall and anal fin skeletal muscle. C. MRI longitudinal section of a male grunion, showing the internal position of the muscular wall of the urogenital papilla relative to the distal intestine (i) and anus (an), its connection to the testes (t), and the urinary duct (u) from the trunk kidney into the urogenital papilla; v = vertebral column. The dark area indicated by * is an artifact of the MRI scan in the region containing the gas bladder. In D, the color-coded segmentation is overlain over part of the section in C, with the wall of the urogenital papilla (pink), the sperm duct traced from the testes (blue), the urinary duct traced from the trunk kidney through the urogenital papilla (green), and part of the distal intestine (brown). E shows the 3D rendering of the urogenital papilla from all of the MRI data; segmentation colors as in D.

urinary duct is separate from the papilla but joins it more posteriorly at the transverse position of the anal opening (Fig. 2B, F).

Comparison of longitudinal, transverse, and sagittal sections in the region containing the urogenital papilla in males (Fig. 1C, 2A, B, D-F, 3F, G) with corresponding regions in females (Fig. 2C, 3A-E) indicates that the urogenital papilla in males is larger and more muscular than the oviduct. When sections were viewed at higher magnification (e.g., Fig. 2E, F, 4B), sperm were visible within the sperm duct of the papilla, and tissues comprising the papilla could be identified. The asymmetrical muscular wall of the urogenital papilla (Fig. 2F, 3F, 4A) was found to contain, in addition to smooth muscle, striated skeletal muscle fibers (Fig. 4C) which were absent in the wall of the oviduct. The presence of skeletal muscle in the wall of the urogenital papilla, but not the oviduct, was confirmed in sections stained with Mallory's trichrome (not shown).

Taken together, our data show that males of the California grunion have a urogenital papilla, dorsal to the intestine and terminating posterior to the anus, a morphological feature not present in females. The females have a less muscular oviduct through which

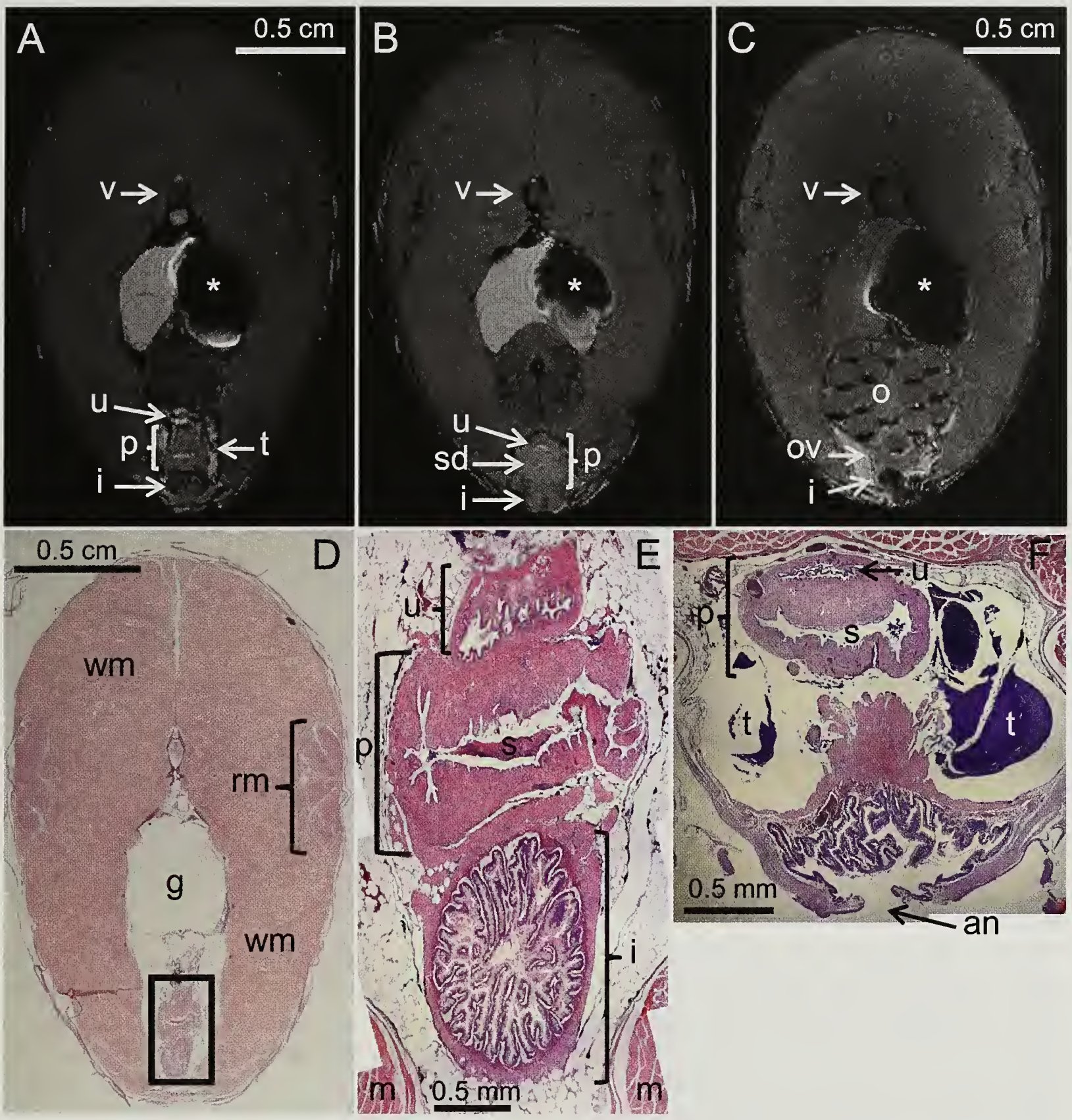

Fig. 2. A, B. MRI images of transverse sections of a male grunion at two positions along the body in the region containing the urogenital papilla (p), with A more anterior than B. Scale bar in A also applies to B. In A, the urinary duct (u) is separate from the urogenital papilla, which contains the sperm duct (sd), whereas in B the urinary duct (u) is within the urogenital papilla, which is dorsal to the intestine (i); v = vertebra, t = testis; the dark areas (*) are an artifact of the MRI scan due to the presence of the gas bladder. C. MRI images of transverse sections of female grunion at a position similar to A, with the ovary containing oocytes (o) and oviduct (ov) dorsal to the intestine (i). D. Transverse histological section of a male in the region corresponding to A, stained with hematoxylin and eosin. Gas bladder (g), slow-oxidative (rm) and fast-oxidative (wm) locomotor muscle are indicated. E. Higher magnification of the boxed area in D with the urinary duct dorsal to the papilla containing the sperm duct (s), which is dorsal to the intestine (i); m = skeletal muscle of the body wall. F. Urogenital papilla in the region of the body near that shown in B, with the urinary duct within the urogenital papilla, and the intestine ending at the anal opening (an); t = paired testes.

oocytes pass as they are deposited in the sand and then fertilized by males during beach spawning. During oviposition, individual females are partially buried in wet sand and are surrounded by multiple males at the sand surface (Thompson 1919; Walker 1952; personal observations). Based on genetic analysis of egg clutches in the sand, which contain

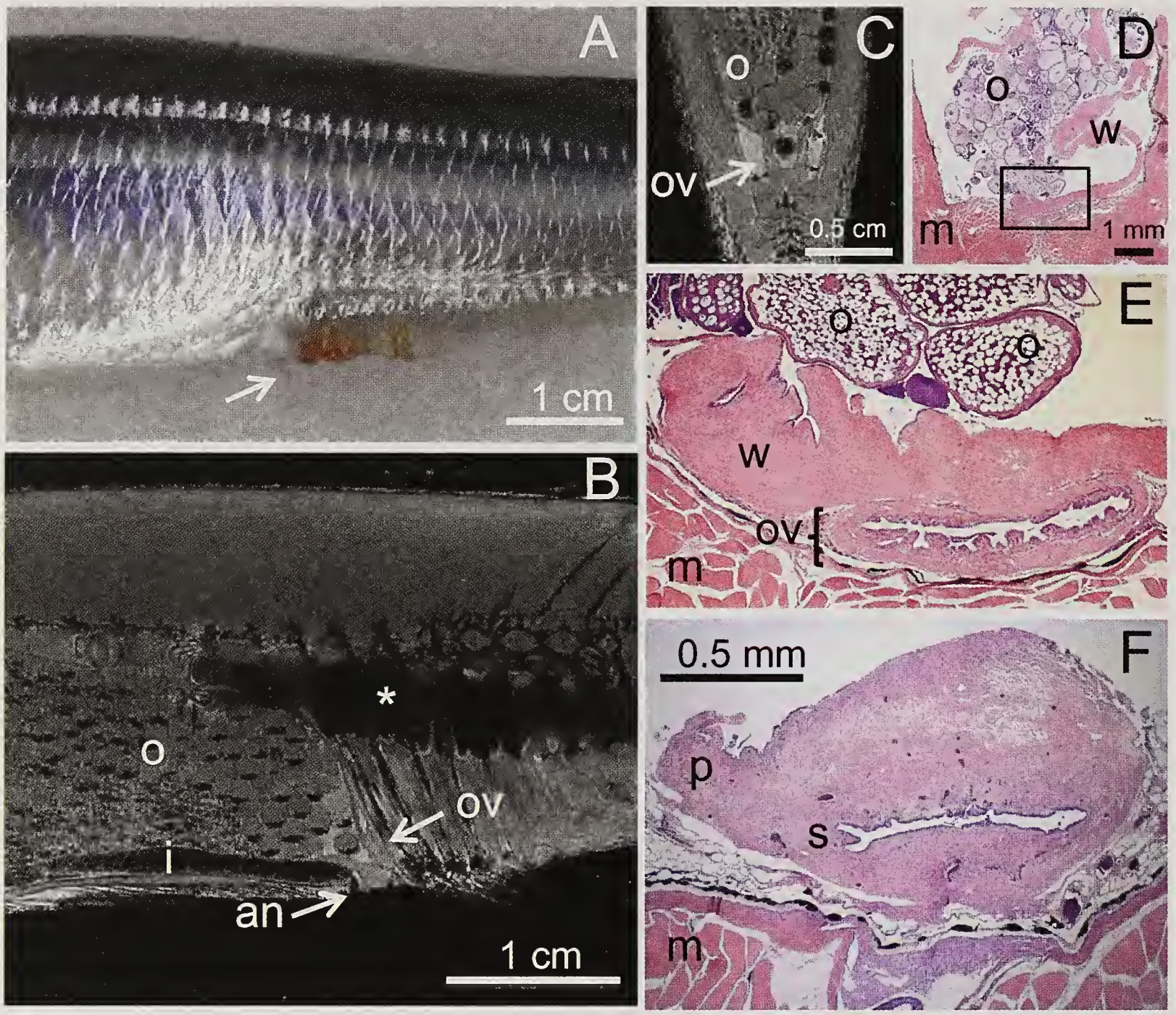

Fig. 3. A. Lateral view of the midbody region of a female California grunion. Arrow points to the genital opening through which oocytes are released, with no protruding structure evident. In A and B, anterior of fish is to the left. B. MRI image of a longitudinal section of a female grunion, showing part of the ovary containing oocytes (o), the oviduct (ov), the distal intestine (i) and the anus (an). The dark area indicated by * is an artifact of the MRI scan in the region containing the gas bladder. C. MRI image of female sagittal section showing the ovary (o) and an oocyte in the oviduct (ov). In C-F, the top of the image is anterior in the fish. D. Histological sagittal section similar to C, stained with hematoxylin and eosin, showing oocytes (o) within the ovary wall (w), a section through the oviduct (box magnified in E), and the surrounding body musculature (m). E. Higher magnification of the boxed area in D, showing the ovary wall (w) and oviduct (ov) wall which contains smooth but not striated muscle. F. Sagittal section of the male genital papilla in a similar position as in the female in E. Scale bar in F also applies to E.

approximately 1000-3000 eggs (Walker 1952; Martin and Carter 2013), up to nine males may fertilize the eggs of one female (Byrne and Avise 2009). There is, therefore, some degree of sperm competition in this species, and we propose that the urogenital papilla allows males to release sperm in a more directed, high-pressure stream to increase the chances of successful fertilization of eggs that a female has deposited in the sand.

Genital and urogenital papillae have been described in numerous fish species, including other members of the Series Atherinomorpha and Order Atheriniformes, of which the California grunion is a member (Dyer and Chernoff 1986; Parenti 2005; Evans and Meisner 2009; Fishelson et al. 2013). In many of these other atherinid fishes, including members of the genus *Labidesthes*, the only other member of the Family Atherinopsidae in which a male urogenital papilla has been described, the papilla is used as an intromittent

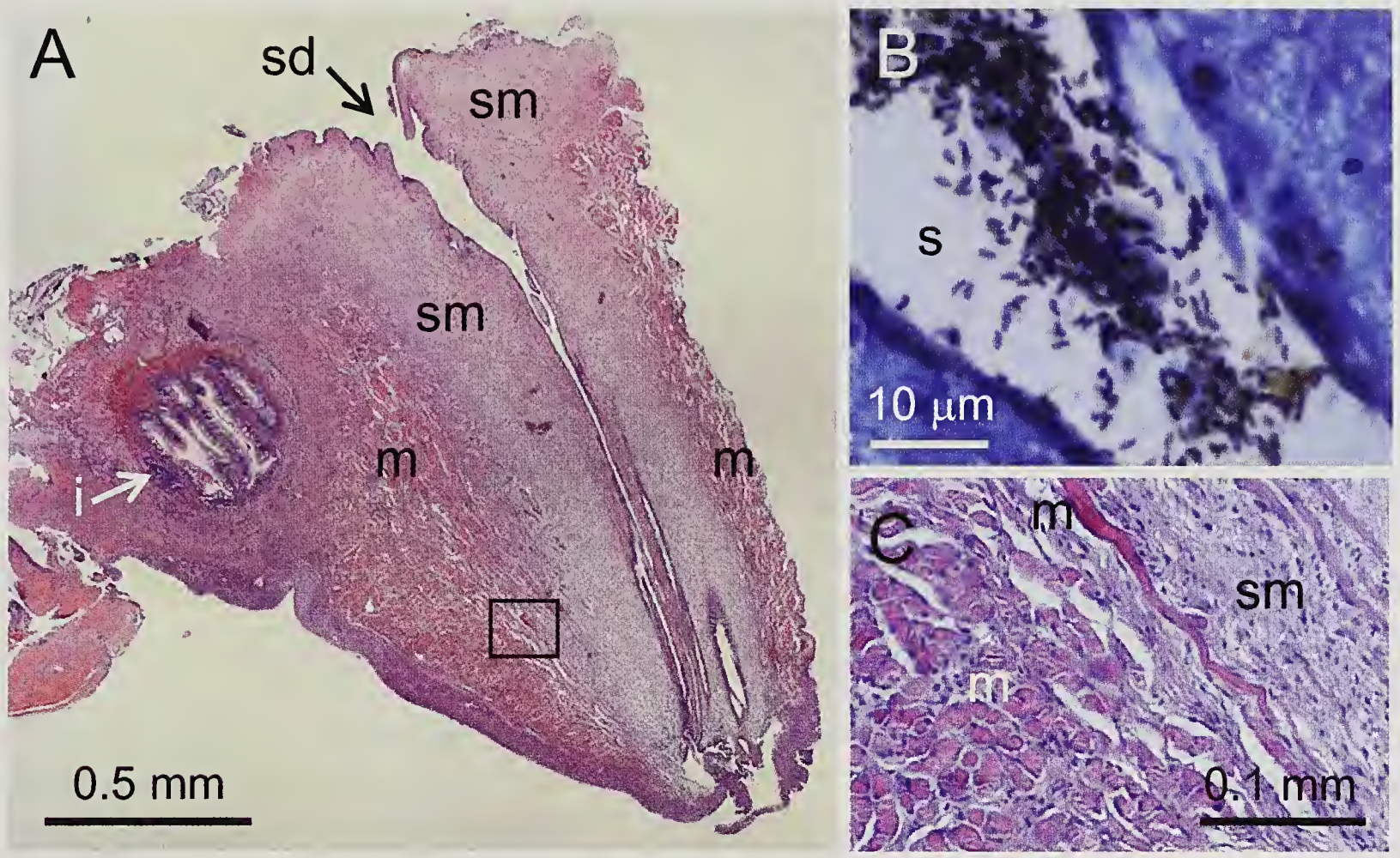

Fig. 4. A. Longitudinal section of whole isolated urogenital papilla and a section of the intestine (i) near the anus, stained with hematoxylin and eosin. Anterior of fish is to the left. The sperm duct (sd) is surrounded by layers of smooth muscle (sm) and striated skeletal muscle (m). B. Higher magnification of part of the sperm duct, showing sperm (s) within the lumen. C. Higher magnification of the black boxed area in A, showing striated skeletal muscle fibers within the urogenital papilla wall.

organ for internal fertilization (Grier et al. 1990; Werneke and Armbruster 2015). In several teleost species, females have a genital papilla that is used as an ovipositor to release oocytes that are then fertilized externally. There are numerous other teleost species in which males release sperm via a urogenital papilla to fertilize eggs externally, including many gobies and blennies (e.g., Har 2000; Ferreira et al. 2010; Fishelson et al. 2013). In the cichlid *Herichthys minckleyi*, "sneaker" males use the urogenital papilla to externally fertilize eggs while another male and female are involved in courtship and oviposition (Oldfield et al. 2015). Rasotto and Shapiro (1998) described the urogenital papilla in males of the bluehead wrasse, *Thalassoma bifasciatum* (Bloch, 1791), which is histologically similar to that of the California grunion and is used in external fertilization during group spawning in this coral-reef sequential hermaphrodite. Rasotto and Shapiro (1998) proposed that the muscular urogenital papilla of male bluehead wrasse allows control of sperm release for multiple, sequential spawning events in a given day. It is possible that the urogenital papilla in the California grunion serves a similar function by limiting sperm release so that males can return to the beach and spawn on the same night or on sequential nights of a spring tide series. If males but not females spawn multiple times during a tide series, this behavior may explain the male-biased sex ratio reported in California grunion samples collected during a given night's spawning event (Thompson 1919; Walker 1949; Santos et al. 2018) and increase a male's chances to fertilize the eggs of more than one female.

To our knowledge, the present study is the first description of a sexually dimorphic characteristic, the urogenital papilla, that can be used to distinguish male and female California grunion externally, and the first study to apply MRI to characterize the three-dimensional structure of a urogenital papilla in any fish species.

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Bull. Southern California Acad. Sci.
118(2), 2019, pp. 109–110
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First Authenticated Record of the Goldspotted Sand Bass, *Paralabrax auroguttatus* from California Waters

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We report on the first authenticated record of the goldspotted sand bass, *Paralabrax auroguttatus* Walford, 1936, in California marine waters (Fig. 1). The fish, a mature female, was 50.0 cm TL (48 cm FL, 42.2 cm SL), and was taken on 30 July 2018, on a rocky reef (34°20.730′N, 119°36.671′W) at a depth of 46 m, off Santa Barbara, California. Fishes captured on the same reef included *Sebastes auriculatus* (brown rockfish), *Sebastes caurinus* (copper rockfish), *Sebastes miniatus* (vermilion rockfish), and *Ophiodon elongatus* (lingcod).

We determined the identity of the specimen using the characters highlighted by Robertson and Allen (2015) as differentiating this species from other serranids: "numerous golden-orange to brownish spots, back with 2–3 thin pale stripes; lining of gill cavity orange" and a "third [dorsal] spine greatly elevated, over three times longer than second." In addition, meristic counts of our specimen are within those listed in Love and Passarelli (in press; Table 1). A second tropical *Paralabrax*, *Paralabrax loro* Walford, 1936, also lives off Mexico. However, we excluded this species because *P. loro* has a series of dark bars on the flanks and an oval white blotch under the posterior dorsal spines and these are lacking in *P. auroguttatus*. In addition, *P. loro* lacks the pale, thin dorsal stripes of *P. auroguttatus* (Robertson and Allen 2015). This specimen is deposited at the Los Angeles Museum of Natural History (LACM 59462-1).

Paralabrax auroguttatus was first described from Guaymas, Mexico (Walford 1936). It grows to at least 71 cm FL (Fitch and Schultz 1978) and inhabits reefs at depths of at least 2–183 m (5–600 ft) (min.: SIO 65-296; max.: M. L., unpubl. data). Before this capture, *P. auroguttatus* was known from just north of Punta Rosalia (28°40′N, 114°16′W) (M. L., unpubl. data) and Isla Cedros, central Baja California (Ramírez-Valdez et al. 2015) to the Gulf of California (Eschmeyer and Herald 1983) and to central Mexico (Robertson and Allen 2015).

We note that there is one previous likely documented record of this species from California marine waters. In the 1976 addendum to Miller and Lea (1972), John Fitch described the capture of a 45.7 cm [listed as 18 in] TL fish taken on a commercial passenger fishing vessel in 1975 [date in Fitch and Schultz 1978], perhaps near Redondo Beach, southern California, in about 61 m [listed as 200 ft in the addendum]. While neither the specimen nor a photograph of the specimen were available to Mr. Fitch, he considered that the description given him by the vessel operator to "fit no other species."

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Fig. 1. A goldspotted sand bass, *Paralabrax auroguttatus*, taken on 30 July 2018, on a rocky reef at a depth of 46 m, off Santa Barbara, California at 34°20.730′N, 119°36.671′W.

Table 1. Meristics of Goldspotted Sand Bass, *Paralabrax auroguttatus* Walford, 1936, from this study and from Love and Passarelli (2019).

Source	Dorsal Fin	Anal fin	Pectoral Fin	Gill rakers 1st Arch
This study	X, 14	III,7	16	6+17
Love and Passarelli (2019)	X,13–14	III,7	14–16	6+17

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Bull. Southern California Acad. Sci.
118(2), 2019, pp. 111–138
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Status of Endangered La Graciosa Thistle *Cirsium scariosum* var. *loncholepis* (Asteraceae) in Coastal Southern California

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Abstract.—La Graciosa thistle *Cirsium scariosum* var. *loncholepis* (Asteraceae) is a biennial or short-lived perennial plant (usually 10 to 100 cm tall) that is endemic to southwestern San Luis Obispo County and western Santa Barbara County in coastal southern California, and little has been published regarding it. The taxon was listed as threatened under the California Endangered Species Act in 1990 and endangered under the U.S. Endangered Species Act in 2000. At Federal listing in 2000, La Graciosa thistle was known from 17 occurrences, 8 of which were likely extirpated. As of 2019, it is known from 21 occurrences of which five are extant, 15 are likely extirpated and 1 has unknown status. La Graciosa thistle exists as groups of individuals in wetland habitats in an arid and semi-arid landscape, and the five extant occurrences are associated with the Callender Dunes and Guadalupe Dunes in San Luis Obispo County. The plants flower once and then die, with a probable life span of 2 to 6 yr. Seed dispersal is by wind and also likely by water, and the taxon appears to have only a minimally persistent soil seed bank. The primary threat to La Graciosa thistle in 2019 is reduced water/lack of water, with groundwater decline as the likely major cause, along with hydrological alteration, drought and climate change. The groundwater decline appears to result primarily from extraction for urban, agricultural and industrial uses, and it is exacerbated by drought and climate change. In 2019, La Graciosa thistle meets the IUCN criteria for endangered.

La Graciosa thistle *Cirsium scariosum* var. *loncholepis* is in the family Asteraceae, along with asters, sunflowers and other thistles, with a geographic range in San Luis Obispo and Santa Barbara Counties, California (Keil 2012). Petrak (1917) described and named *C. loncholepis* from a specimen collected in 1906 "near La Graciosa," which is now in south Orcutt in northwest Santa Barbara County (Wilken 2009 in USFWS 2018). *Cirsium loncholepis* was listed as threatened under the California Endangered Species Act in 1990 (California Department of Fish and Wildlife [CDFW] 2017b) and endangered under the U.S. Endangered Species Act in 2000 (U.S. Fish and Wildlife Service [USFWS] 2000). It is recognized as a 1B.1 rare plant (seriously endangered) by the California Native Plant Society.[1]

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[1] California Native Plant Society. 2018. *Cirsium scariosum* var. *loncholepis*. *In*: Inventory of Rare and Endangered Plants of California. Available (13 July 2018): http://www.rareplants.cnps.org/detail/487.html

Kelch and Baldwin (2003) studied phylogeny of the genus *Cirsium*, which includes ~60 species in North America. Although many species are well differentiated, some are poorly differentiated due to introgression or incipient speciation. Keil (2006) synonymized *C. loncholepis* with *C. scariosum* var. *citrinum*. However, Keil (2010) subsequently recognized *C. scariosum* var. *loncholepis* as a new combination and variety. Although Keil observed few morphological features that distinguish the two taxa, he reported the coastal populations of *C. scariosum* var. *loncholepis* and montane populations of *C. scariosum* var. *citrinum* are separated by 150 km with no intervening population, and with substantial ecological differences. Keil (2012) referred to *C. scariosum* as a variable complex of intergrading races, and with some plants not readily assignable to any of its six varieties. He stated that *C. scariosum* var. *loncholepis* is probably derived from populations of *C. scariosum* var. *citrinum* near the headwaters of the Cuyama River, which is a major tributary of the Santa Maria River. The largest occurrence of *C. scariosum* var. *loncholepis* is downriver at the mouth of the Santa Maria River. *Cirsium scariosum* var. *loncholepis* is the same listed entity as *C. loncholepis*, and with the same morphological characteristics and geographic distribution (USFWS 2011).

At Federal listing in 2000, La Graciosa thistle was reported to occur from southern Monterey County southward to the Santa Ynez River in Santa Barbara County (~150 km), and from the Pacific Ocean inland to Orcutt (16 km). The plant was known from 17 occurrences, eight of which were likely extirpated. Identified threats were hydrological alterations including groundwater extraction in/near the Guadalupe Dunes (most serious threat), energy-related activities in the dunes that modify habitat (maintenance, hazardous waste cleanup), uncontrolled cattle grazing in the dunes and along the Santa Maria River, commercial development in the dunes (a proposed sea port), extensive loss of habitat, and invasive species (USFWS 2000). Our purpose is to review and enhance the knowledge of La Graciosa thistle, in particular its distribution, ecology, abundance, threats, management and conservation status in 2019.

Materials and Methods

We surveyed, censused, mapped and monitored many occurrences of La Graciosa thistle from 2005 to 2019, and we identified two new occurrences. We considered unpublished reports and other documents in files of the USFWS and CDFW, information in the California Natural Diversity Database, personal communications with other knowledgeable persons, and the sparse published literature. Using all available information, we summarize the body of knowledge of La Graciosa thistle, including its distribution, known occurrences, ecology, abundance, threats, management and conservation status in 2019. We considered a location with the taxon as a separate occurrence if it is >0.4 km from the nearest occurrence (California Department of Fish and Game [CDFG] 2011). We determined latitude/longitude and elevation with a global positioning system device in the field or with Google Earth aerial imagery. Latin and common names of plants follow Baldwin et al. (2012). Stated areas of properties are from records of the Counties of San Luis Obispo and Santa Barbara. In Appendix 1, we discuss each occurrence according to its assigned number in the California Natural Diversity Database (CDFW 2017). The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the USFWS.

Fig. 1. Upper left: La Graciosa thistle *Cirsium scariosum* var. *loncholepis* in flower near the mouth of the Santa Maria River in the Guadalupe Oil Field, San Luis Obispo County, California, 4 April 2015 (occurrence 6). Upper right: A vegetative individual of La Graciosa thistle near the mouth of the Santa Maria River in the Guadalupe Oil Field, 21 March 2017. Lower left: A young vegetative individual of La Graciosa thistle near the mouth of the Santa Maria River in the Guadalupe Oil Field, 21 March 2017. Lower right: Achenes (dry fruit with one seed only) of La Graciosa thistle from a plant at Big Twin Lake on the private property of Dune Lakes Limited, San Luis Obispo County, California, 30 August 2017 (occurrence 11).

Results and Discussion

Review of La Graciosa Thistle.—La Graciosa thistle is a biennial or short-lived perennial plant that flowers once and then dies (Lea 2002; Teed 2003; Keil 2012), with a probable life span of 2 to 6 yr (Lea). It is an erect or spreading mound-like plant with spines on the leaves and flower heads (Fig. 1). Plants have one or more stems that are usually 10 to 100 cm tall. The lower leaves are 10 to 30 cm long with spiny petioles (leaf stalks) and usually deeply lobed with secondary lobes or teeth. The leaves have wavy edges, with the bases of the middle and upper leaves forming short, spiny wings along the petioles. Flower heads are 2 to 4 cm wide in tight clusters and at tips of stems. Flowers are 25 to 30 mm long and nearly white with a purplish tube containing purple anthers. The fruits are achenes (dry fruit containing one seed only), 3 to 4 mm long, and topped with a pappus (umbrella of small hairs) 15 to 25 mm long (Keil and Turner 1993) that facilitates wind dispersal. La Graciosa thistle can be confused with clustered thistle *C. brevistylum* (Table 1) and cobwebby thistle *C. occidentale*.

La Graciosa thistle exists as groups of individuals in wetland habitats in an arid and semiarid landscape. The plants inhabit the margins of wetlands (swales, lakes, ponds, freshwater marshes, streams, rivers, seeps) in southwestern San Luis Obispo County and

Table 1. Fourteen previously recognized occurrences of La Graciosa thistle *Cirsium scariosum* var. *loncholepis* that were removed from the California Natural Diversity Database (K. Lazar, CDFW, Sacramento, pers. comm. 2017). SBA Co = Santa Barbara County, SLO Co = San Luis Obispo County.

Occurrence	General location	Reason for removal from California Natural Diversity Database
5	Vandenberg Air Force Base, SBA Co	misidentified clustered thistle *Cirsium brevistylum*
7	Santa Maria River	included in occurrence 6
9	Jack Lake, SLO Co	combined into occurrence 8
15	Santa Maria River	included in occurrence 6
17	Surprise Lake, SLO Co	mis-mapped, same as occurrence 12
21	Vandenberg Air Force Base, SBA Co	misidentified clustered thistle
22	Vandenberg Air Force Base, SBA Co	misidentified clustered thistle
23	Vandenberg Air Force Base, SBA Co	misidentified clustered thistle
24	Vandenberg Air Force Base, SBA Co	misidentified clustered thistle
25	Vandenberg Air Force Base, SBA Co	misidentified clustered thistle
26	Vandenberg Air Force Base, SBA Co	misidentified clustered thistle
27	Vandenberg Air Force Base, SBA Co	misidentified clustered thistle
29	Laguna Lake Natural Reserve, SLO Co	misidentified Chorro Creek bog thistle *C. fontinale* var. *obispoense*
35	Rancho Guadalupe Dunes Co. Park, SBA Co	technical error or misidentified clustered thistle

western Santa Barbara County in coastal southern California (Fig. 2; Table 2). The majority of occurrences ($n = 11$) are associated with wetlands in the backdunes of two coastal sand dune complexes: the Callender Dunes just south of the city of Arroyo Grande, and the contiguous Guadalupe Dunes just north of the Santa Maria River. Many of the wetlands here occur where the groundwater table is at or near the surface (Lea 2002; CDFG 2005), and the water levels rise and fall naturally with rainfall. Low water levels can be exacerbated by drought (Holland et al. 1995 in USFWS 2018). Normal annual rainfall in the geographic range is ~330 to 430 mm (mean 380 mm; California Department of Water Resources 2004), but 241 mm was recorded in 2014 (Luhdorff and Scalmanini 2015 in USFWS 2018). La Graciosa thistle blooms from April to September (Keil 2012). Potential pollinators include ants, beetles, bees, butterflies and flies (Keil 2001 in USFWS 2018; Lea 2002). Seed dispersal is by wind (Keil and Turner 1993; Lea 2002; USFWS 2016) and also likely by water.

Three important studies on the life history of La Graciosa thistle were conducted by Hendrickson (1990 in USFWS 2018), Lea (2002) and Teed (2003). Hendrickson counted density of seedlings in occurrence 6 at the mouth of the Santa Maria River in the Guadalupe Oil Field (1,093 ha) in San Luis Obispo County. During March there were up to 352 individuals per m^2, but during August there were no more than 64 individuals per m^2. Shaded seedlings had substantially larger leaves than those in sunlight. Hendrickson wondered if the soil contained dormant seeds that could buffer population fluctuations. She attempted to germinate seedlings from soil samples of the Santa Maria River bed, but none emerged. She concluded that no dormant seeds were in the soil samples, or that some mechanism prevented any dormant seeds from germinating.

Lea (2002) studied population dynamics and demography of two occurrences (6, 18) of La Graciosa thistle in the Guadalupe Oil Field that represent two extremes of habitat conditions. The two groups of studied plants were separated by 2.6 km. The plants in

Table 2. Conservation status of the 21 known occurrences of La Graciosa thistle *Cirsium scariosum* var. *loncholepis* in San Luis Obispo and Santa Barbara Counties, California. SBA Co = Santa Barbara County, SLO Co = San Luis Obispo County.

Occurrence	Location	Landowner	Protected	Status at Federal listing 2000	Status 2018	Last year seen	Last year searched
1	S of Lompoc-Surf Rd 4.8 km airline SE of Surf, SBA Co	Vandenberg Air Force Base	no	likely extirpated	likely extirpated	1958	2008
2	E Branch of Cañada de las Flores, SBA Co	private	no	likely extirpated	likely extirpated	1989	2009
3	N Branch of Cañada de Las Flores, SBA Co	private	no	likely extirpated	likely extirpated	1989	2009
4	S Orcutt, SBA Co	unknown	no	likely extirpated	likely extirpated	1906	2017
6	Santa Maria River bed + floodplain near river mouth, SLO Co + SBA Co	two private including Chevron Corp	currently partially	extant	extant, decreased number	2019	2019
8	Jack Lake + unnamed wetland 556 m N, SLO Co	private Phillips 66 Company	no	unknown	likely extirpated	1998	2017
10	Mud Lake, SLO Co	private with conservation easement to Land Conservancy SLO Co	yes	likely extirpated	likely extirpated	1983	2017
11	Big Twin Lake, Small Twin Lake + Hospital Lake	private with conservation easement to Land Conservancy SLO Co	yes	extant	extant, increased number	2019	2019
12	Surprise Lake, SLO Co	Oceano Dunes State Vehicular Recreaton Area	yes	extant	in decline, near extirpation	2019	2019
13	NW shore of Oso Flaco Lake, SLO Co	Oceano Dunes State Vehicular Recreation Area	yes	likely extirpated	likely extirpated	1990	2015
14	Pismo State Beach, NW Oceano, SLO CO	Pismo State Beach	yes	likely extirpated	likely extirpated	1969	2017
16	Black Lake, SLO Co	Land Conservancy SLO Co	yes	likely extirpated	likely extirpated	1970	2017
18	NW monitoring well M-11, Guadalupe Oil Field, SLO Co	private Chevron Corp	currently	extant	extant, decreased number	2018	2018

Table 2. Continued.

Occurrence	Location	Landowner	Protected	Status at Federal listing 2000	Status 2018	Last year seen	Last year searched
19	immediately N of Santa Maria River, 602 m NNW of 8th St (Guadalupe), SLO Co	private	no	unknown	likely extirpated	1991	2017
20	Lettuce Lake + unnamed wooded swale 749 m W of Jack Lake, SLO Co	Oceano Dunes State Vehicular Recreation Area	yes	likely extirpated	likely extirpated	1990	2017
28	roadside, West Main Street extended, S Guadalupe, SBA Co	private	no	unknown	likely extirpated	1983	2017
30	946 m SW of Oso Flaco Lake, SLO Co	Oceano Dunes State Vehicular Recreation Area	yes	unknown	likely extirpated	1967	2017
31	3 Pond West, SLO Co	Guadalupe-Nipomo Dunes National Wildlife Refuge	yes	extant	in decline, near extirpation	2019	2019
32	Entrance Ponds, Guadalupe Oil Field + adjoining private property, SLO Co	two private including Chevron Corp	currently partially	extant	likely extirpated	2014	2018
33 (new)	Price Ranch Rd, NE of Los Alamos, SBA Co	private	no	unknown	unknown	1975	1975
34 (new)	Santa Maria River bed, SLO Co + SBA Co	two private including Chevron Corp	currently partially	extant	likely extirpated	2006	2018

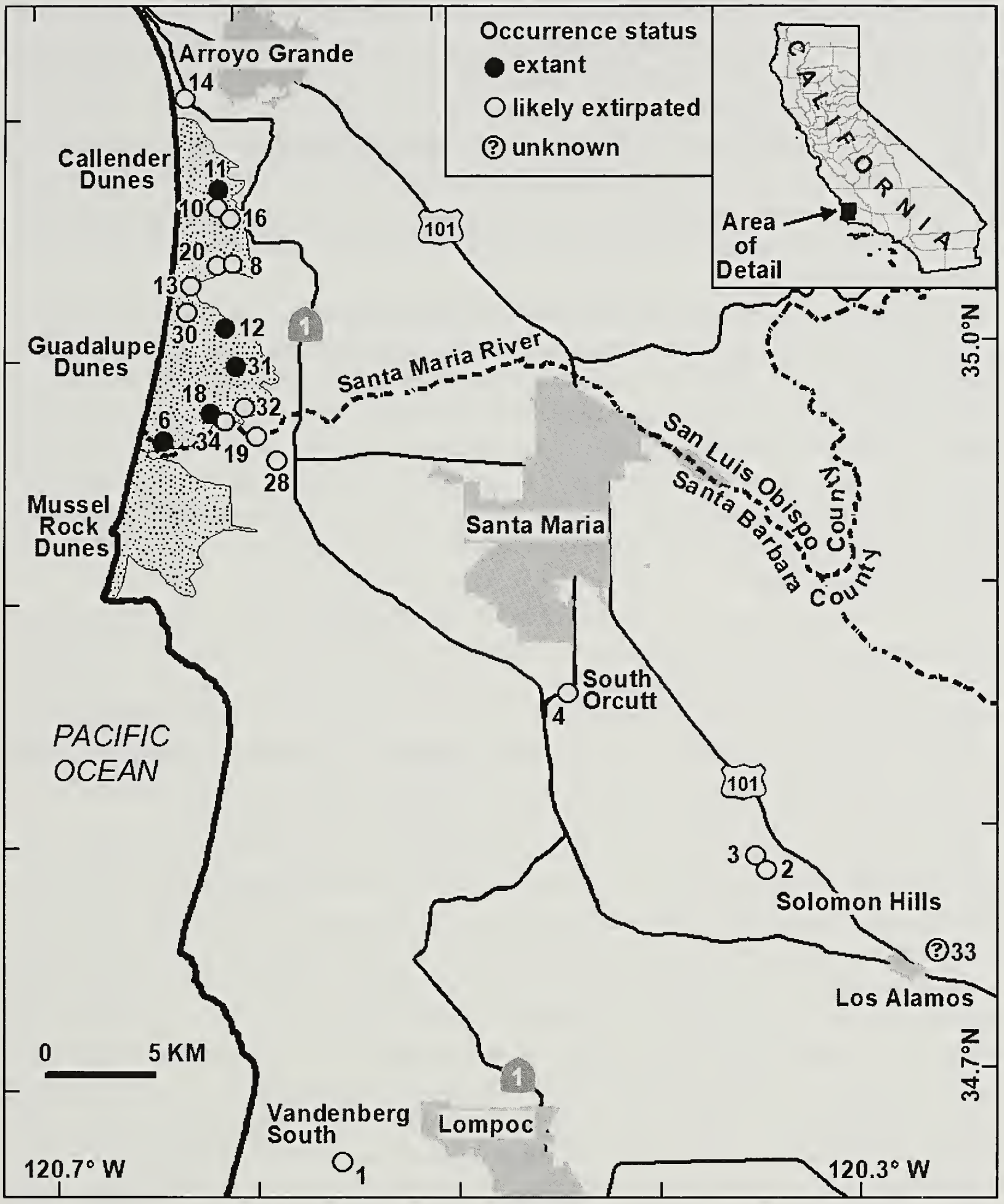

Fig. 2. Geographic distribution of the 21 known occurrences of La Graciosa thistle *Cirsium scariosum* var. *loncholepis* in San Luis Obispo and Santa Barbara Counties, California. We tentatively recognize four geographic populations, which from north to south are Sand Dune Complexes (16 occurrences), South Orcutt (1 occurrence), Solomon Hills (3 occurrences), and Vandenberg South (1 occurrence).

occurrence 6 were at the mouth of the Santa Maria River (moister conditions, 0.6 km inland), while the plants in occurrence 18 were further inland (drier conditions, 2.8 km inland). The plants with moister conditions had larger leaves than plants with drier conditions. Further, the majority of plants that flowered (85%) were in the larger size classes in each group: moister conditions, 85% with largest leaf >10 cm length; and drier conditions, 92% with largest leaf >7 cm length. All flowering plants soon died, indicating a

monocarpic species. At both occurrences the larger flowering plants produced more seeds than smaller flowering plants (larger plant mean = 473 seeds per plant; smaller plant mean = 168 seeds per plant; Lea in USFWS 2008). March had the greatest number of germinating plants, and seedlings comprised the greatest portion of the study sample. The germination rate in seeded plots was 13% (occurrence 18), and control plots had only few wild seedlings emerge from any resident soil seed bank (Lea 2002). Herbivory by snails was observed. The population growth rate for both occurrences was >1, which indicated plant numbers should be increasing if parameters and ecological conditions remained the same as during the study.

Teed (2003) studied ecology and population demography of the same two occurrences as Lea (2002). The majority of seeds were produced by the largest plants at both occurrences. Insects damaged 19% of flower heads at occurrence 6 and 38% at occurrence 18. Like Lea (2002), Teed reported that all flowering individuals soon died. Most wild seedlings originated from seeds deposited the previous summer rather than from a resident soil seed bank. In addition, Teed concluded that La Graciosa thistle is adapted to periodic disturbance. Further, she suspected the clumped pattern within its habitat is likely due to seeds being deposited near the parent plant and where the microclimate is especially favorable for survival and growth.

Of the 13 occurrences of La Graciosa thistle for which zero plants were recorded and then a subsequent census conducted, 11 also had zero plants recorded in any subsequent census (Table 3). In other words, only two of 13 occurrences were proven to be extant after a census record of zero plants. This, combined with the observations of Hendrickson (1990 in USFWS 2018), Lea (2002) and Teed (2003), appears to show that La Graciosa thistle has only a minimally persistent soil seed bank. Therefore, any occurrence that has not had flowering plants over several consecutive years is at high risk of extirpation.

The 21 known occurrences of La Graciosa thistle are in the following drainages, from north to south: Meadow Creek (1 occurrence), Arroyo Grande Creek (3 occurrences), Oso Flaco Creek (5 occurrences), Santa Maria River (8 occurrences), San Antonio Creek (3 occurrences), and Santa Ynez River (1 occurrence). The plant requires habitat with water on or near the surface, and the largest occurrence is associated with extensive wetlands at the mouth of the Santa Maria River at the edge of the Guadalupe Dunes. La Graciosa thistle grows in moist sandy soil with or without an upper organic layer in open areas with sun or shade (Hendrickson 1990 in USFWS 2018). Hendrickson observed that most La Graciosa thistle among the sand dunes were on level areas usually at the eastern or northern edges of a swale or lake. The western edges of these wetlands are often bordered by steep slopes of sand because the strong prevailing winds are from the northwest (USFWS 2016; Hunt 1993 in USFWS 2018). La Graciosa thistle is frequently associated with willows *Salix* (Hendrickson 1990 in USFWS 2018). The historical distribution of La Graciosa thistle likely included the former extensive wetlands in the middle and lower Santa Maria River Valley. In particular, the former extensive wetlands near Orcutt have been drained for agriculture and urban use (CDFG 2005). Also, many extensive wetlands previously associated with the lower Santa Maria River have been replaced with intensive agriculture, including its former route to Oso Flaco Lake that existed prior to 1860 (Cooper 1967; Hunt 1993 in USFWS 2018).

La Graciosa thistle has $2n = 34$ chromosomes (Ownbey et al. 1975). Villablanca (2012 in USFWS 2018) studied genetic diversity of La Graciosa thistle at three locations (occurrences 6, 18, 32) in the Guadalupe Oil Field. Genic diversity (average heterozygosity per locus) was high in each occurrence, inferring one genetic population with historic

Table 3. Approximate numbers (flowering + vegetative individuals) of La Graciosa thistle *Cirsium scariosum* var. *loncholepis* in the 21 known occurrences in San Luis Obispo and Santa Barbara Counties, California. Column 6C is the portion of occurrence 6 on property of Chevron Corporation, which includes 22% of the mapped area on two private properties. X = present. Occurrences 33 and 34 are new. The data are from USFWS (2018) unless otherwise indicated, although much of that data is ours.

	Occurrence																			
Year	1	2	3	4	6C	8	10	11	12	13	14	16	18	19	20	28	30	31	32	33	34
2019									14[4]									25[6]			
2018					902[4]			X[5]	2-4[4]	0[4]			748[4]					5[4]	0[4]		0[4]
2017				0	535	0	0	≥245	65		0	0	913	0	0	0	0	≥5	0		
2016					1,510				37				803[1]						0		
2015					2,833	0			1	0			5,374[1]		0				0		
2014					2,880								23,590[1]					10	1		
2013					1,612				37				2,812[1]			0		172	12		
2012					953								649[1]						18		
2011					1,654								247[1]						35		
2010					4,464								240					300	34		
2009		0	0		9,751								280						23		
2008	0				8,362				10				1,005					X	94		
2007	0	0	0		1,339								1,558					50	119		
2006					1,746								2,240						280		<10
2005																0			420		
2002					X								X								
2001					X								X								
2000					X								X								
1998						2			7	0					0						
1997									54									87			
1995	0				X								31								
1994	0												X								
1993																					
1991														100							
1990	0	0	0	0	23,320[2] (106,002[3])	>112			29	34	0	0	137		≥12						
1989		X	X																		
1988								20	25												

Table 3. Continued.

	Occurrence																				
Year	1	2	3	4	6C	8	10	11	12	13	14	16	18	19	20	28	30	31	32	33	34
1987	0	X	X						50												
1986	0	20	5	0	X	X	0	X	0	0	0	0									
1985		X	X		X																
1984		X	X		X			11-50													
1983		X	X		X		>100					0				X					
1982	0																				
1981					X	X	1-10	4-40	11-50												
1980						X				X					X						
1979						≥30															
1977					X																
1976											0										
1975																X					
1974					X																
1973		X	X					X								X					
1969											X										
1970												X									
1968						X				X											
1967																	X				
1964						X															
1962					X					X											
1960										X											
1958	X							X													
1949	X									X											
1910											X										
1906				X																	

[1] Numbers for 2011 to 2016 include outplantings in restored area.

[2] A prorated number (22%) for the 26 ha of occurrence 6 on private property of Chevron Corporation.

[3] Hendrickson (1990 in USFWS 2018) estimated 106,002 individuals for the entire occurrence 6 (118 ha) on two private properties.

[4] pers. obs.

[5] L. Roddick, *Land Conserv. San Luis Obispo Co.*, Calif., pers. comm. 2018.

[6] D. Kirkland, *USFWS*, Ventura, *Calif.*, pers. comm. 2019.

connectivity among the three occurrences. In addition, the diversity values implied a large breeding population for each occurrence. This suggested either the numbers of individuals in the vegetative state (not flowering) were smaller than numbers in the soil seed bank, or the size of the ancestral population was substantially larger than the size of the current population. In light of the reports by Hendrickson (1990 in USFWS 2018), Lea (2002) and Teed (2003), and our own observations in Table 3, Villablanca's alternative is inferred: that is, the ancestral population was substantially larger than the current population. There was no evidence that the most interior of the three occurrences (32) was more differentiated than the other two occurrences. However, only occurrence 6 (mouth of Santa Maria River) showed significant gene flow, in which 23% of reproducing individuals were migrants and 70% were locals. Occurrences 18 and 32 showed no significant gene flow, with the vast majority of all individuals in these two occurrences being non-migrants and, therefore, likely derived in situ. These two occurrences seemed to be demographically independent of any other occurrences, although with some genetic connections. Occurrences 18 and 32 were shown to be inbreeding, which we suspect is due to loss of connectivity across the landscape. Inbreeding is a threat because it increases the extinction risk of small populations. In particular, the impact of environmental stress can become significantly greater at higher levels of inbreeding (Bijlsma et al. 2000). Occurrence 32 is now likely extirpated, with zero plants recorded each year from 2015 to 2018 (Table 3).

In 2019, a total of 21 extirpated and extant occurrences of La Graciosa thistle ranges coastally from Pismo State Beach (occurrence 14), San Luis Obispo County, southward to the floodplain of the Santa Ynez River west of Lompoc (near south entrance of Vandenberg Air Force Base; occurrence 1) in Santa Barbara County (50 km distance), and from the coast inland to a freshwater marsh 1.6 km northeast of Los Alamos (occurrence 33), Santa Barbara County (32 km distance). This comprises an area of 626 km^2. The occurrence previously reported in Monterey County has been determined to be Alameda County thistle *C. quercetorum* (Lea 2002). Most occurrences ($n = 16$) are in or proximal to the Callender Dunes and Guadalupe Dunes. The majority ($n = 17$) are within 6.3 km of the coast, while four are inland at substantially greater distances. Elevations for the coastal occurrences are ≤43 m, whereas the inland occurrences are at 107 to 190 m. USFWS (2004) identified the primary constituent elements of critical habitat for La Graciosa thistle: moist sandy soils associated with dune swales; margins of dune lakes and marshes, seeps, intermittent streams and river margins; native plant communities that support the associated native wetland species, including rush *Juncus*, tule *Scirpus* and willow; and hydrologic processes that support the favored soil moisture regime, particularly a stable groundwater table near the surface.

In coastal southern California, the wind is capable of eroding sand so deep that groundwater is uncovered, and this has produced a scattering of small wetlands throughout the dunes. A coastal dune swale is a scoured depression between sand dunes where the bottom is at or near the groundwater table. The plant communities here are dominated usually by phreatophytes (deep-rooted woody plants that depend upon shallow groundwater within or near reach of their roots; Culver and Lemly 2013), in particular native arroyo willow *Salix lasiolepis* (Williams 1989; pers. obs.). Further, native marsh baccharis *Baccharis glutinosa* and the invasive herb poison hemlock *Conium maculatum* (both with deep roots; Robinson 1958; Rains et al. 2004) are indicators of potential microhabitat for La Graciosa thistle within the wetlands. The recent drought has caused a drop in groundwater levels, and just several centimeters can make a substantial difference to the plant community. During drought years, lakes within the dunes have dried when groundwater was lowered

by pumping (USFWS 2016; Holland et al. 1995 in USFWS 2018; Kofron 2019). One effect of drought on La Graciosa thistle may be suppression of seedling recruitment (USFWS 2016) because seedlings have less tolerance for drier conditions than larger plants (Huber 2005).

Of the 21 known occurrences, five are extant in 2018 (occurrences 6, 11, 12, 18, 31), 15 are likely extirpated, and one has unknown status. The five extant occurrences are on private and public lands and with varying protection: one on private property of Chevron Corporation (occurrence 18) currently protected by a USFWS (2005) biological opinion issued under the U.S. Endangered Species Act; one on private properties of Chevron Corporation and another landowner (occurrence 6) with current protection on Chevron property by a USFWS biological opinion; one protected on private property by a conservation easement to Land Conservancy of San Luis Obispo County (occurrence 11); one protected on Oceano Dunes State Vehicular Recreation Area (occurrence 12); and one protected on Guadalupe-Nipomo Dunes National Wildlife Refuge (occurrence 31). The details and status of the 21 occurrences are provided in Appendix 1, two of which are new (occurrences 33, 34). In addition, we determine that occurrence 35 should be removed from the California Natural Diversity Database because it appears based on technical error or misidentification. At Federal listing in 2000, all occurrences except one were believed to be on private land (USFWS 2000), seven occurrences were reported to have <60 plants each, and numbers for the largest (occurrence 6) were reported to fluctuate from 6,000 to 54,000 plants.

The primary threat to La Graciosa thistle in 2019 is reduced water/lack of water, with groundwater decline as the likely major cause, along with hydrological alteration, drought and climate change. The groundwater decline appears to result primarily from extraction for urban, agricultural and industrial uses, and it is exacerbated by drought and climate change. Groundwater decline causes habitat loss and degradation for La Graciosa thistle. Past development and agriculture have caused substantial habitat loss and fragmentation by conversion of wetlands to other uses (Hendrickson 1990 in USFWS 2018). From the 1850s to 1987, 90% of California's coastal wetlands disappeared (Caughman and Ginsberg 1987). In the 21st century, the remaining wetlands in coastal California continue to decrease in quantity and quality (USFWS 2011).

Seventeen occurrences of La Graciosa thistle are in the Santa Maria Valley Groundwater Basin, which extends from Pismo Beach to Point Sal (south of mouth of Santa Maria River) and from the coast inland to east of the cities of Santa Maria and Orcutt (Luhdorff and Scalmanini 2015 in USFWS 2018). Recharge occurs primarily by seepage from the major streams, rainfall percolation and subsurface flow (California Department of Water Resources 2004). In general, groundwater levels in the region fluctuate naturally over time but within a relatively small range (Bartolino and Cunningham 2003). Conversely, from 1918 to 1975, groundwater volume in the basin declined by 33%, including 44% declines in the Guadalupe and Nipomo storage units and a 59% decline in the Santa Maria storage unit (Morro Group 1990 in USFWS 2018). These three groundwater units underlie or are proximal to 15 of the 21 known occurrences of La Graciosa thistle, and they provide most of the water for the intensive agriculture (primarily vegetables and strawberries; Luhdorff and Scalmanini in USFWS 2018), residential development, urbanization and industry immediately east of these occurrences. Groundwater decline from extraction can lower the groundwater table so that wetland plants can no longer survive (Bartolino and Cunningham; Alley et al. 1999), and we suspect this is likely a primary cause for decline of La Graciosa thistle (Oyler et al. 1995 in USFWS 2018).

From the late 1960s to 2002, the Santa Maria Valley Groundwater Basin alternately experienced substantial decline and recharge. Since 2002, groundwater levels gradually declined because of land uses along with a lack of water releases from Twitchell Dam on the Cuyama River (48 km upriver from mouth of Santa Maria River), which released no water in 10 of 15 yr between 2002 and 2016 and only limited releases in most other years and 2017 along with extended drought (Anderson 2016 in USFWS 2018; Luhdorff and Scalmanini 2015 in USFWS 2018; R. Gonzalez, Bureau of Reclamation, Fresno, Calif., pers. comm. 2017, 2018). The dam is operated to maximize groundwater recharge for the basin, and it provides some water flow for the Santa Maria River. However, in the vicinity of the Guadalupe Oil Field, the river would usually be dry if not for runoff irrigation wastewater (Mock 2000 in USFWS 2018). In 2014 at the southern edge of the Nipomo Mesa, groundwater level was highest in March but by October had declined 7 m, which seems likely linked to seasonal agricultural irrigation (Luhdorff and Scalmanini in USFWS 2018). In addition, discussions ensued regarding the sale of water in Twitchell Reservoir to the city of Montecito (San Luis Obispo Coastkeeper and Los Padres Forest Watch 2017 in USFWS 2018), which is east of the city of Santa Barbara in southern Santa Barbara County. The Santa Maria Valley Management Area is a subarea of the basin, and it encompasses 11 occurrences of La Graciosa thistle, including four of the five extant occurrences. Groundwater levels here have fluctuated substantially since the 1920s, with widespread decline between 1945 and late 1960s, including declines of 6.1 to 12.2 m near the coast and 21.3 m near Orcutt. These declines resulted by progressively increasing urban and agricultural demands, along with drier climatic conditions (Luhdorff and Scalmanini in USFWS 2018).

The Dune Lakes comprise 10 natural lakes in the Callender Dunes between the city of Arroyo Grande and Oso Flaco Lake: Willow Lake, Pipeline Lake, Celery Lake, Hospital Lake, Big Twin Lake, Small Twin Lake, Bolsa Chica Lake, White Lake, Mud Lake and Black Lake. They are relatively shallow, with Black Lake the deepest at 2.4 m in 2017 (L. Roddick, Land Conserv. San Luis Obispo Co., Calif., pers. comm. 2018). These lakes are 1.6 km inland and near the western edge of Nipomo Mesa, and they contain three occurrences of La Graciosa thistle: occurrence 10 at Mud Like (likely extirpated); occurrence 11 at Big Twin Lake, Small Twin Lake and Hospital Lake (extant); and occurrence 16 at Black Lake (likely extirpated). Land uses on the mesa include urban and residential, cultivated farmland, three golf courses and industry, especially the ConocoPhillips Santa Maria Refinery. Four water companies extract groundwater from beneath the mesa, and many private properties have their own water wells (Nipomo Mesa Management Area Technical Group 2017 in Kofron 2019). Nine lakes have recently dried, one which was dry for 5 yr (2012 to 2017). The best available information points to a combination of two primary causes for drying of the Dune Lakes (Kofron 2019): first, extensive groundwater extraction beneath Nipomo Mesa to the east; and second, severe drought. Hydrological alteration by State Highway 1 and a railroad constructed across the lower end of Black Lake Canyon is possibly a third but minor contributing cause. The lakes are on private property of Dune Lakes Limited, with exception of Black Lake on private property of Land Conservancy of San Luis Obispo County. In addition, the conservancy holds a conservation easement over the other nine lakes. The property manager of Dune Lakes Limited wishes to restore several lakes in partnership with government agencies and the non-profit sector.

The Entrance Ponds (2.5 ha) are a wetland in the Guadalupe Oil Field at its western edge, and it contains occurrence 32 of La Graciosa thistle (Fig. 3), which we monitored annually from 2005 to 2018. The number of plants declined rapidly here from 420 in 2005 to zero in 2015, 2016, 2017 and 2018. The primary threats here include lack of water by

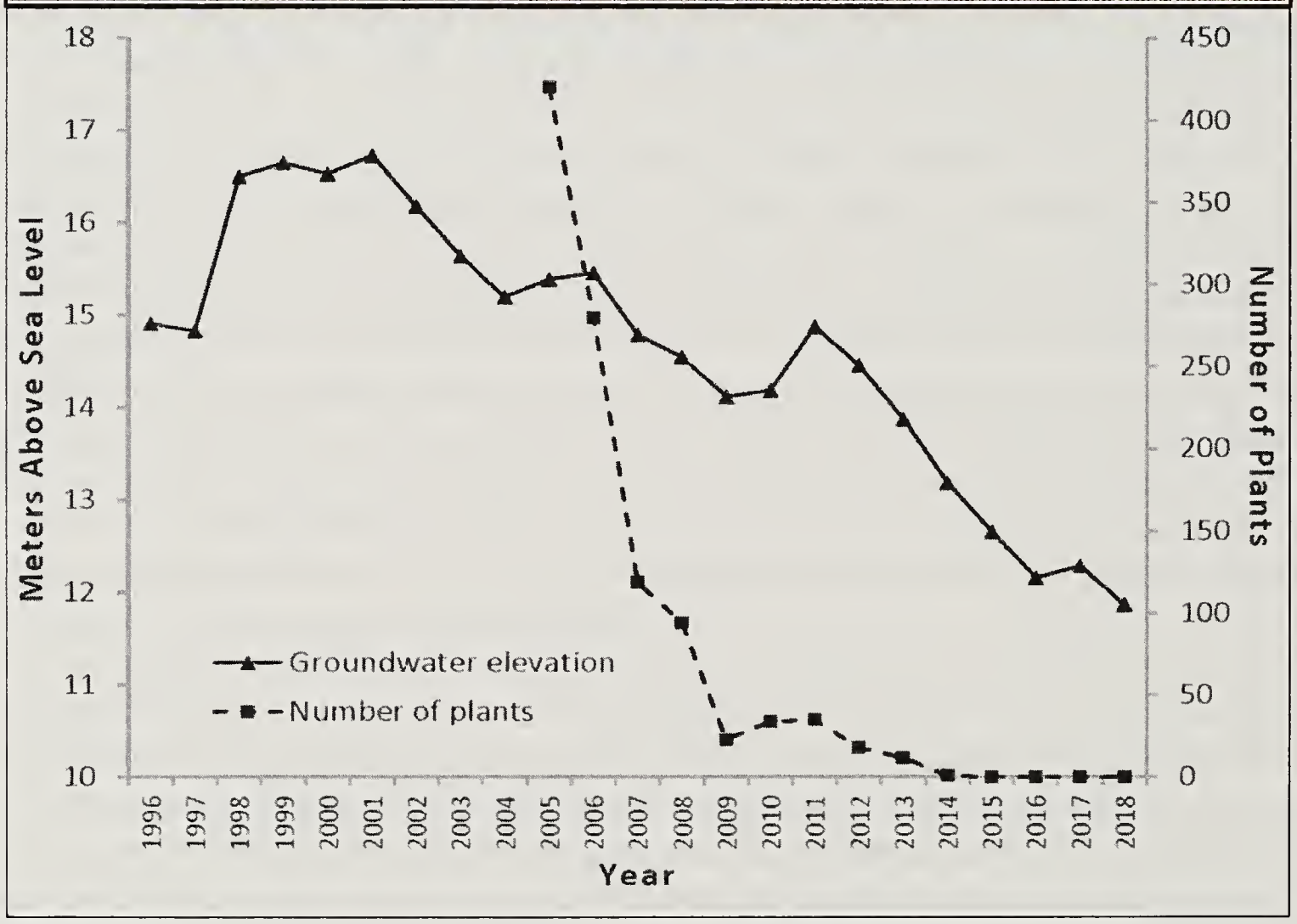

Fig. 3. Upper: Occurrence 32 of La Graciosa thistle *Cirsium scariosum* var. *loncholepis* at the Entrance Ponds, Guadalupe Oil Field, San Luis Obispo County, California, 6 September 2017. The wetland comprises two swales, one on private property of Chevron Corporation and one on the adjacent private property. This occurrence is likely extirpated, having quickly declined from 420 plants in 2005 to 0 in 2015, 2016, 2017 and 2018. In 2017 the wetland was forested with arroyo willow *Salix lasiolepis*, and many had died and collapsed. In 2001 the groundwater table was at the surface, and as of 2018 it had declined 4.86 m. Lower: The relationship between the groundwater table and La Graciosa thistle at the Entrance Ponds in the Guadalupe Oil Field, San Luis Obispo County, California (occurrence 32). Elevation (meters above mean sea level) of the groundwater table was measured in October of each year at monitoring well 35W-6A1, which is 180 m southeast of occurrence 32. In October 2001 the groundwater elevation was 16.73 m, whereas in October 2018 it was 11.87 m, which is a drop of 4.86 m. The Entrance Ponds and monitoring well 35W-6A1 connect to the same aquifer. La Graciosa thistle is a wetland plant, and the occurrence is now likely extirpated.

Table 4. Current threats to the five extant occurrences of La Graciosa thistle *Cirsium scariosum* var. *loncholepis* in 2019.

	Occurrence				
Threats in 2019	6	11	12	18	31
Reduced water/lack of water	X	X	X	X	X
Groundwater decline	X	X	X	X	X
Groundwater extracton	X	X	X	X	X
Drought	X	X	X	X	X
Climate Change	X	X	X	X	X
Hydrological alteration	X	X		X	
Herbivory			X		X
Invasive plant species	X	X	X		
Flooding	X				
Dead vegetation altering habitat		X	X		X
Feral pigs	X		X		
Gophers	X			X	
Inbreeding				X	
Agriculture	X				
Uncontrolled cattle grazing	X				
Road and ditch maintenance	X				
Stochastic events	X	X	X	X	X

groundwater decline and drought, invasive plants (Italian plumeless thistle *Carduus pycnocephalus*, ripgut brome *Bromus diandrus*, foxtail fescue *Vulpia myuros*), and fallen dead arroyo willow trees altering the habitat. The dead arroyo willow are consistent with a drop in the groundwater table (Holland et al. 1995 in USFWS 2018; Alley et al. 1999; D. Chipping, Calif. Polytech. St. Univ., San Luis Obispo, pers. comm. 2017). Monitoring well 35W-6A1 is 180 m southeast of the Entrance Ponds, and both connect to the same aquifer. In October 2018, the elevation of groundwater at the well was 11.87 m, whereas in October 2001 it was 16.73 m. The groundwater table here declined 4.86 m over the 17 yr. The Entrance Ponds are no longer a wetland, and the wetland plants including La Graciosa thistle have disappeared.

Species with small geographic ranges are vulnerable to extinction by climate change (Allan et al. 2005). La Graciosa thistle is now extant only in vicinity of the Callender Dunes and Guadalupe Dunes. In consideration of the life history traits used by Anacker et al. (2013), La Graciosa thistle is highly vulnerable to climate change because of its wetland habitat specialization. In particular, California is becoming hotter and drier. The summers of 2017 and 2016 were each the warmest in California since record keeping began in the late 1800s.[2] Considering data up to 2015, Brown et al. (2016 in USFWS 2018) reported that most of the warming occurred in the past 35 yr, with 15 of the 16 warmest years occurring since 2001. The 3-yr period from 2012 to 2014 was the hottest and driest in California in the 100-yr time frame considered by Mann and Gleick (2015), and it was the most severe drought in California in the past 1,200 yr (Griffin and Anchukaitis 2014). Both anomalous precipitation and temperature have contributed to development of the

[2] NOAA National Centers for Environmental Information. 2018. Climate at a glance: U.S. time series. average temperature. Available (12 February 2018): https/www.ncdc.noaa.gov/cag/time-series/us

Table 5. Previously reported threats to the 21 known occurrences of La Graciosa thistle *Cirsium scariosum* var. *loncholepis* in San Luis Obispo and Santa Barbara Counties, California. The data are from USFWS (2018) and Kofron (2019).

Occurrence	Status	Previously identified threats
1	likely extirpated	reduced water, flooding, agriculture, vegetation management, invasive species, hydrological alteration, drought, climate change
2	likely extirpated	drought, agriculture, cattle grazing, overgrazing, feral pigs, climate change
3	likely extirpated	drought, agriculture, cattle grazing, overgrazing, feral pigs, climate change
4	likely extirpated	agriculture, development, drought, climate change
6	decreased number, large occurrence 2018	oil production, development, herbivory, cattle grazing, cattle trampling, flooding, feral pigs, iceplant, gophers, drought, climate change, groundwater decline, groundwater extraction
8	likely extirpated	herbivory, invasive species, off road vehicles, lack of water, drought, reproductive failure, climate change, groundwater decline, groundwater extraction
10	likely extirpated	unstable water level, manipulation of water level, vegetation management, lack of water, accumulation of dead bulrushes, groundwater decline, groundwater extraction, hydrological alteration, drought, climate change
11	increased number, small occurrence 2017	manipulation of water level, vegetation management, lack of water, accumulation of dead bulrushes, invasive species, groundwater decline, groundwater extraction, hydrological alteration, drought, climate change
12	in decline, near extirpation 2019	drought, flooding, herbivory, reproductive failure, willow expansion, off road vehicles, hunters, feral pigs, lack of water, habitat alteration by fallen trees, climate change, groundwater decline, groundwater extraction
13	likely extirpated	off road vehicles, drought, reproductive failure, willow expansion, climate change, groundwater decline, groundwater extraction
14	likely extirpated	iceplant, invasive species, drought, climate change
16	likely extirpated	reduced water, unstable water level, bulrushes, groundwater decline, groundwater extraction, hydrological alteration, drought, climate change
18	decreased number, large occurrence 2018	lack of water, drought, cattle grazing, cattle trampling, invasive species, gophers, herbivory, climate change, groundwater decline, groundwater extraction
19	likely extirpated	agriculture, invasive species, drought, climate change, groundwater decline, groundwater extraction
20	likely extirpated	flooding, off road vehicles, herbivory, willow expansion, *Rubus*, lack of water, habitat alteration by fallen trees, drought, climate change, groundwater decline, groundwater extraction
28	likely extirpated	agriculture, development, road and ditch maintenance, drought, climate change, groundwater decline, groundwater extraction
30	likely extirpated	vegetation management, drought, climate change, groundwater decline, groundwater extraction
31	in decline, near extirpation 2019	cattle trampling, feral pigs, lack of water, herbivory, drought, climate change, groundwater decline, groundwater extraction
32	likely extirpated	cattle grazing, cattle trampling, lack of water, drought, invasive species, habitat alteration by fallen trees, groundwater decline, climate change, groundwater extraction
33 (new)	unknown	drought, climate change
34 (new)	likely extirpated	invasive species, cattle grazing, drought, climate change, groundwater decline, groundwater extraction

severe multiyear drought in California. However, precipitation (specifically, the lack of) is the dominant driving force while temperature acts to moderately accentuate the drought (Luo et al. 2017). Wetland water levels are lowered by drought (USFWS 2016) and, because La Graciosa thistle is restricted to wetlands, a severe drought could substantially reduce or eliminate its habitat.

In 2018 a recovery team (relevant land managers, university botanist, USFWS) was formed for La Graciosa thistle, with the immediate goal to prevent extinction and any further extirpations. At its first meeting in September 2018, the team agreed to immediately implement annual monitoring at each extant occurrence for 5 yr, along with vegetation and herbivore management and consideration for supplemental watering and outplantings. In addition, a seed head will be collected from each extant occurrence for seed banking. Further, consideration should be given for re-establishing several extirpated occurrences where threatening factors can be reduced. Regrettably, however, these actions may not be enough to conserve La Graciosa thistle unless the groundwater decline is reversed.

Conclusions.—In 2019, we identify reduced water/lack of water as the primary threat to La Graciosa thistle, with the likely major cause being groundwater decline primarily from extraction for urban, agricultural and industrial uses, along with hydrological alteration, drought, and climate change. The current threats to the extant occurrences are shown in Table 4, and the previously reported threats are shown in Table 5. Using our information and international standards (IUCN 2012, 2014), La Graciosa thistle in 2019 meets the IUCN criteria for endangered. Specifically, the extent of the present occurrence (24 km^2) and the area of occupancy (<1.6 km^2) of La Graciosa thistle are small, in continuing decline, and comprise only five known extant occurrences. The quality of the habitat is continuing to decline. The most recent counts of plants at the five extant occurrences in 2017 to 2019 are 902 (for 22% of mapped area of occurrence 6), 748, ≥245, 25, and 14. Considering these attributes, La Graciosa thistle faces an extremely high risk of extirpation at four of the five extant occurrences.

Acknowledgements

The following persons assisted in the field: David Chipping of California Polytechnic State University, Lindsey Roddick of the Land Conservancy of San Luis Obispo County, Melissa Kelly of Rancho Guadalupe Dunes County Park, Ronnie Glick of Oceano Dunes State Vehicular Recreation Area, Tom Applegate of Wildwing Consulting, and Debora Kirkland and Kristie Scarazzo of USFWS. Mark Elvin of USFWS helped with startup of the project, and Robert Taylor and Mark Metevier of USFWS prepared the map. Katie Gross and Kristi Lazar of CDFW provided information from the California Natural Diversity Database. Tom Olson of Gracia and Associates, Danielle Wold of Chevron Corporation and Michael Peña of the City of Guadalupe provided logistical support. Steve Madsen and Robert Castellaños allowed access to the private properties that they manage, and they also assisted in the field. We discussed genetics with Francis Villablanca of California Polytechnic State University. Without the contributions of all these persons, this project could not have been accomplished. Finally, we thank David Keil of California Polytechnic State University for reviewing and commenting on the manuscript.

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Appendix 1

The 21 known occurrences of La Graciosa thistle *C. scariosum* var. *loncholepis* in San Luis Obispo and Santa Barbara Counties, California.

Occurrence 1. 34.662962, -120.556957 (Wilken et al. 2008 in USFWS 2018); 34.669904, -120.558230; 9 to 10 m elevation; 4.8 km east of Surf (both sides of road to Lompoc), Vandenberg Air Force Base, Santa Barbara County. The coordinates are a best estimate. The first pair of coordinates is documented by specimens (C.F. Smith 5820) collected in 1958 that are now in multiple herbaria: California Academy of Sciences, California Polytechnic State University, Harvard University, Rancho Santa Ana Botanic Garden, San Diego Natural History Museum, Santa Barbara Botanic Garden, University of California Riverside, and University of Minnesota. Smith (1958 in USFWS 2018) wrote "scattered in saline soil along edge of willows south of Lompoc-Surf Rd. about three miles airline southeast of Surf. El. 100 ft.?" Despite many searches (1982, 1986, 1987, 1990, 1994, 1995, 1998, 2007, 2008; USFWS 2018), La Graciosa thistle has not been recorded here since 1958. Hendrickson (1990 in USFWS 2018) reported the occurrence as extirpated.

She described Smith's location as a willow thicket at the edge of a cultivated field, with thick weeds (mainly invasive blessed milkthistle *Silybum marianum*) at the edges. Hendrickson suggested that a spraying program targeting invasive bull thistle *Cirsium vulgare* may have extirpated the occurrence. Hendrickson spoke with Smith regarding the location, and then she searched "both sides of the drainage along the base of the hills near Union Ave. almost to the VAFB South Gate." The second pair of coordinates is based on CAS413797 in the California Academy of Sciences (Consortium of California Herbaria 2017 in USFWS 2018), which was collected in 1949 with the following data: on north side of railroad, Camp Cooke Military Reservation (now Vandenberg Air Force Base), about 3 miles east of Surf, on road to Lompoc, south side of Santa Ynez River, over 10-acre field. We viewed aerial imagery (dated July 2016) of the location using Google Earth. It is in the south floodplain of the Santa Ynez River on Vandenberg Air Force Base near its south entrance at the junction of West Ocean Avenue and Arguello Boulevard. Much of the area has been cleared and disked for agriculture, along with the construction of buildings and parking areas. However, some natural areas remain along the unnamed tributary of the Santa Ynez River. The Bradbury Dam is on the Santa Ynez River (71 km upriver), and it opened in 1958. This dam altered the hydrology downstream (Schmalzer et al. 1988) and likely adversely affected habitat of La Graciosa thistle. The occurrence is 4.8 km inland.

Occurrence 2. 34.784740, -120.345300; 34.784709, -120.341174; 176 and 180 m elevation; east branch of Cañada de Las Flores marsh, Solomon Hills, Santa Barbara County. The coordinates are from Elvin (2007 in USFWS 2018) and Hendrickson (1990 in USFWS 2018). This occurrence is documented by several specimens collected in 1973 that are at California Polytechnic State University, Santa Barbara Botanic Garden and University of California Santa Barbara. The specimen labels state collection in a freshwater marsh on the east branch of Cañada de Las Flores, in open coast live oak *Quercus agrifolia* woodland, northwest of Los Alamos. This occurrence is on one private property (272 ha) and immediately south of the private property with occurrence 3 (same landowner). Although the former landowner J. Sainz (Thornton 2008 in USFWS 2018) showed one site for occurrence 2 on her map, Hendrickson showed two sites including one at the "eastern end of the valley" that was identified by on-site discussion with Sainz. This occurrence is 26 km inland and in the headwaters of San Antonio Creek watershed. We viewed aerial imagery (dated February 2018) of the location using Google Earth. The marsh is undeveloped, however, most land adjacent to the tributary to San Antonio Creek is under crop agriculture. We could not obtain permission to visit this occurrence in 2017. This occurrence is likely extirpated. Additional information is provided under occurrence 3.

Occurrence 3. 34.790550, -120.347120; 34.789301, -120.345593; 184 and 186 m elevation; north branch of Cañada de las Flores marsh, Solomon Hills, Santa Barbara County. The coordinates are from Elvin (2007 in USFWS 2018) and Hendrickson (1990 in USFWS 2018). This occurrence is documented by two specimens collected in 1973 that are at Santa Barbara Botanic Garden and University of Minnesota. This occurrence exists as two sites on one private property (135 ha), and it is immediately north of the property with occurrence 2. We refer to this occurrence as north branch of Cañada de las Flores marsh. The label of one specimen states collection in a seep on a hillside in Cañada de Las Flores, in open coast live oak woodland northwest of Los Alamos. Google Earth aerial imagery (February 2018, June 2017) showed both sites were recently disked and a paved road divides the two sites. This occurrence is 25 km inland and in the headwaters of San Antonio Creek watershed. We could not obtain permission to visit this occurrence in 2017. This occurrence is likely extirpated.

Smith (1976, 1998) reported the location for occurrences 2 and 3 as Cañada de las Flores marsh near Los Alamos. Hendrickson (1990 in USFWS 2018) saw no plants in 1990, but observed the habitat with the landowner J. Sainz. Hendrickson wrote the following regarding the species and its habitat: around seeps in hillsides above freshwater marsh, the two main sites are on open west-facing slopes near seeps with grassland on the slopes above, and two secondary sites (one near oak woodland and one near willows at eastern end of valley), population has declined in recent years, possibly extirpated. Sainz stated the numbers fluctuate every year, however, she had never known them to be completely absent. She estimated there were usually ~15 plants at each of the four locations. Later, Sainz (Thornton 2008 in USFWS 2018) wrote that generally she never observed more than two or three plants in any location, and typically no more than nine to twelve plants in any year, and the plants were on the edge of the water. Further, she stated the plant was never abundant, and it did not appear every year. The only herbarium specimens were collected in 1973, and photographs documented presence in 1987 (Thornton 2008 in USFWS 2018). Elvin (2007 in USFWS 2018) visited the occurrences with Sainz in 2007 and saw no plants. He reported that habitat conditions had declined due to grazing intensity, but the essential habitat components remained (freshwater seeps, native vegetation). Kisner (2009 in USFWS 2018) visited the occurrences with Sainz in 2009 and saw no plants, although habitat remained at three sites.

Occurrence 4. 34.858748, -120.440081; 110 m elevation; south Orcutt, Santa Barbara County. This occurrence is based solely on the holotype (CAS165) of La Graciosa thistle collected by Eastwood in 1906. The specimen label states "Flora of the Country Adjacent to Santa Maria, California, near La Graciosa." Smith (1976, 1998) suggested the location to be near the mouth of San Antonio Creek on Vandenberg Air Force Base, but the plant has never been found there. Hendrickson (1990 in USFWS 2018) believed the specimen is from near the former community of La Graciosa, which was forcibly abandoned and burned in the 1870s. She determined the village location to be 600 m southwest of the junction of East Clark Avenue and State Highway 135 in south Orcutt, which is now mostly freeway and urban development. Similarly, Couch and Fuhring (2010 in USFWS 2018) stated that the former community of La Graciosa was near Graciosa Station (Pacific Coast Railway; Rand McNally and Company 1897), which was 1.6 km south of Orcutt Station (Clark Street in Orcutt), and that it was probably on the site now occupied by the junction of Highway 1 and State Highway 135. Further, Wilken (2009 in USFWS 2018) studied Eastwood's itinerary and other relevant details. Likewise, he concluded the specimen is probably from near the former Graciosa Station or from near the former village of La Graciosa in what is now Orcutt (specifically, near the junction of East Clark Avenue and State Highway 135). Therefore, we accept that the holotype is from south Orcutt. The town of Orcutt is contiguous with and immediately south of the city of Santa Maria. Searches for the plant in Orcutt in 1986 (McLeod 1986 in USFWS 2018) and 1990 (Hendrickson) were negative. McLeod observed "there were a couple of likely sites along the Pine Canyon branch of Orcutt creek near Rice Ranch Road but no La Graciosa thistle." South Orcutt is on a terrace of Orcutt Sand (a soil type), and Pine Canyon is a short canyon on the north slope of Graciosa Ridge (Woodring and Bramlette 1950). Hendrickson stated that, although extensive wetlands previously existed in the Orcutt area and could have provided habitat, they have been drained and are now fields or developed. She considered the occurrence to be extirpated. We viewed aerial imagery (dated January 2015) of the relevant areas in south Orcutt using Google Earth in March 2017, and they are mostly developed. However, small stretches of Pine Canyon and the creek to the west of south Orcutt are undeveloped, although adjacent to development. We searched in Pine Canyon in Orcutt Community Park in 2017 with negative results. We recommend that searches occur in the creek to the west of south Orcutt. This occurrence is not protected, and it is likely extirpated. The location is 16 km inland.

Occurrence 6. 34.962550, -120.635704; 3 to 12 m elevation; both sides of Santa Maria River and its floodplain and river bed extending from near the river mouth (260 m inland from wet beach sand) to 3 km inland (34.968882, -120.615740), San Luis Obispo and Santa Barbara Counties. Our coordinates are the approximate center of the mapped areas (118 ha) shown by Hendrickson (1990 in USFWS 2018), McLeod (1984 in USFWS 2018), and Padre Associates (2017 in USFWS 2018). This is the largest occurrence of La Graciosa thistle, which in 1990 was estimated to comprise 106,002 individuals (Hendrickson). The ecosystem is mostly riparian and with moist soil having high organic content in the upper layers and sandy below (Hendrickson). In 1990, the greatest concentration of plants was nearer the river mouth and associated with native willow thickets, mostly at the south and west margins. Further inland there was less association with willows, and the plants were scattered across the open river bed with a dense cover of native species: marsh jaumea *Jaumea carnosa*, saltgrass *Distichlis spicata*, Pacific silverweed *Potentilla anserina* subsp. *pacifica*, and yerba mansa *Anemopsis californica*. Holland et al. (1995 in USFWS 2018) stated that La Graciosa thistle at the river mouth "occurs around the elevated margins of the marshes on the north side...and to a lesser extent in the riverbed and as understory in riparian woodland." In terms of occupied mapped areas, 22% (26 ha) of the occurrence is in the Guadalupe Oil Field owned by Chevron Corporation, and 78% (92 ha) is on adjacent private property that is used for cattle grazing. The Guadalupe Oil Field is a decommissioned oil field that is undergoing remediation/restoration under a USFWS (2005) biological opinion. The part of this occurrence in the oil field is managed by Chevron, and it is currently protected. Chevron does not allow cattle grazing on this property. In addition, Kelly (2013 in USFWS 2018) reported five individuals of La Graciosa thistle along the drainage ditch on the north side of West Main Street before it enters Rancho Guadalupe Dunes County Park, Santa Barbara County (2.4 km inland), and there were more individuals nearby in the river bed.

We have annually monitored the three occurrences of La Graciosa thistle in the oil field since 2005. Most recently in 2018 we recorded 902 individuals in the Chevron portion of occurrence 6 (pers. obs.), which is the second lowest number since we began monitoring, and it includes several restored/replanted areas. The highest recorded number is 9,751 individuals in 2009. Padre Associates (2017 in USFWS 2018) attributed the lowest number of plants in 2017 (535 individuals) to flooding and prolonged inundation of the habitat, and it appears the occurrence is still recovering. This occurrence previously experienced major flooding during 1998 and incurred damage with substantially decreased numbers of plants

(Lea 2002; Chesnut 1998 in USFWS 2018). We saw no impacts by feral pigs in the oil field in 2016, likely because of trapping by Chevron, and only minor impacts in 2017 (Padre Associates) and 2018 (pers. obs.). We viewed aerial imagery of the relevant areas using Google Earth (dated July 2016) in May 2017, and they were mostly undeveloped. The mapped occupied areas are mostly in the river bottomlands. Water flow in the Santa Maria River is controlled in part by releases from Twitchell Dam, which was constructed in 1957. The dam is 48 km upriver from the Pacific Ocean, and it is operated to optimize groundwater recharge for the Santa Maria Valley Groundwater Basin. Groundwater flow in the basin is generally westward, from inland toward the Pacific Ocean (California Department of Water Resources 2004). The primary threats to occurrence 6 are reduced water (groundwater decline, groundwater extraction, hydrological alteration, drought, climate change), flooding, uncontrolled cattle grazing, invasive plant species, and potentially crop agriculture replacing cattle grazing as a land use.

Potential new locations. 50 m east and perpendicular to south end of road at Santa Maria River mouth (UCSB855597); and south of mouth of Santa Maria River (RSA166700). The two specimens were collected in 1985 and 1962, respectively (Consortium of California Herbaria 2017 in USFWS 2018). The details for both locations are vague but appear to be in Rancho Guadalupe Dunes County Park in Santa Barbara County. We recommend searching for La Graciosa thistle immediately south of the mouth of the Santa Maria River in the park.

Occurrence 8. La Graciosa thistle ($n = 2$) was last observed here in 1998 (Chesnut 1998 in USFWS 2018). We observed no plants in 2017, and the occurrence is now likely extirpated. Based on information in a draft of USFWS (2018), CDFW combined occurrence 9 into occurrence 8 because the two sites are separated by <0.4 km (K. Lazar, CDFW, Sacramento, pers. comm. 2018). However, because there are two separate wetlands, we present the information for each wetland prior to 2018.

Occurrence 8 before 2018. 35.042943, -120.605579; 35.041660, -120.603016; 10 m elevation; west and south margins of unnamed wetland, 0.56 km northwest of Jack Lake, 1.1 km west of ConocoPhillips Santa Maria Refinery, San Luis Obispo County. The coordinates are based on Chesnut (1998 in USFWS 2018). The second location is 250 m east (Hendrickson 1990 in USFWS 2018) of the first location. The occurrence is on private property (43.5 ha; Phillips 66 Company) that is managed by California State Parks (Oceano Dunes State Vehicular Recreation Area). McLeod (1980, 1986 in USFWS 2018) reported plants here in 1980 and 1986. Hendrickson observed 49 plants in 1990. Forty-eight plants were at the west margin of a swale (no water) with willows in dry sandy soil. The plants were in a small area between willows and *Rubus*. Many of the plants had been damaged by herbivores, and shading and crowding/competition with other plants were identified as potential threats. One plant was 250 m east of this group. Chesnut saw two plants in 1998. MIG/TRA Environmental Sciences (2016 in USFWS 2018) reported no plants in 2015, and we saw no plants in 2017. This occurrence is likely extirpated. We viewed aerial imagery of the location using Google Earth (dated July 2016) in March 2017, and it is in the backdunes of the Callender Dunes, which cover most of the Nipomo Mesa (Cooper 1967). The location is 2.4 km inland, and the area appeared to be in a natural state. Although not protected, this occurrence is on property closed to the public.

Occurrence 9 before 2018. 35.038399, -120.602649; 35.037580, -120.600785; 10 m and 13 m elevation; Jack Lake, 672 m west of ConocoPhillips Santa Maria Refinery, San Luis Obispo County. The coordinates are based on Hendrickson (1990 in USFWS 2018) and Chesnut (1998 in USFWS 2018). The occurrence is on private property (143.5 ha; Phillips 66 Company) that is managed by California State Parks (Oceano Dunes State Vehicular Recreation Area). The swale is in the backdunes between vegetated and non-vegetated tall sand dunes. Howald (Lazar, pers. comm. 2018) observed 30 plants at the edge of the swale in 1979, and Hendrickson observed plants at two locations: a small area at the northwest edge of the swale (41 plants plus seedlings), and another area with 22 plants scattered among *Baccharis* at the east margin. Chesnut saw no plants in 1998, MIG/TRA Environmental Sciences (2016 in USFWS 2018) reported no plants in 2015, and we saw no plants in 2017. In 2017 the swale was densely forested with arroyo willow, and many had died and collapsed, which is consistent with a drop in the groundwater table (Holland et al. 1995 in USFWS 2018; Alley et al. 1999; Chipping, pers. comm. 2017). The swale held no water but the soil was damp. Although we searched much of the swale, a large portion was not searched, which presents a possibility that plants were present but not observed. However, the occurrence is likely extirpated. The ecosystem is mostly in a natural state, although the lack of water during March 2017 was a threat. We viewed aerial imagery of the location using Google Earth (dated July 2016) in March 2017, and it is in the backdunes of the Callender Dunes. The location is 2.8 km inland. Although not protected, this occurrence is on property that is closed to the public.

Occurrence 10. 35.062048, -120.610190; 5 m elevation; east margin of Mud Lake, south of Arroyo Grande, San Luis Obispo County. The coordinates are from Howald (1981 in USFWS 2018), who observed

1 to 10 plants in 1981 and first reported this occurrence. The habitat was freshwater marsh bordering a freshwater dune lake, with native arroyo willow, California bulrush *Schoenoplectus californicus*, and spreading gooseberry *Ribes divaricatum*. An herbicide targeting Pacific poison oak *Toxicodendron diversilobum* had been sprayed on parts of the property (491 ha). Turner and Pemberton (1983 in USFWS 2018) reported >100 plants in 1983. McLeod (1986 in USFWS 2018) saw no plants in 1986 and offered several possible reasons: the boundary where the plants grow changes from year to year; the plants may have been affected by pumping water; the plants may have been affected by spraying for Pacific poison oak; or the two previous reports may have been based upon misidentified clustered thistle (California Department of Fish and Game 2005). We visited the lake in August 2017, and it was mostly dry and with its bed covered by a dense layer (30 to 91 cm) of vegetation debris comprised predominantly of stems of dead California bulrushes. The property manager (S. Madsen, Dunes Lake Ltd., Arroyo Grande, Calif., pers. comm. 2017) stated the lake had been dry for 5 yr. We searched the east margin of the lake where the plants were reported to occur and found no La Graciosa thistle. We identify reduced water/lack of water and the accumulation of dead vegetation as threats. Madsen stated that he wishes to restore the lake, including removal of dead vegetation, and he was agreeable to replanting La Graciosa thistle. The provisioning of water other than runoff irrigation wastewater will be a challenge (Kofron 2019). The USFWS (2018) will endeavor to support restoration of the Dune Lakes. We viewed aerial imagery of the location using Google Earth (dated July 2016) in April 2017, and it is in the backdunes of the Callender Dunes. This occurrence is on private property with several houses and other buildings. The terrestrial vegetation is managed for hunting California quail *Callipepla californica*. The location is 2 km inland. The property is zoned for four houses, along with grazing and recreational hunting. The Land Conservancy of San Luis Obispo County has owned a conservation easement over Mud Lake since 2000, and formerly the California Coastal Conservancy and the Nature Conservancy since 1996. Although this occurrence is protected, it is likely extirpated. Occurrence 11 is on this same property.

Occurrence 11. 35.069523, -120.606845; 35.070530, -120.607960; 35.070440, -120.60915; 35.069465, -120.606603; 35.070880, -120.610254; east margin of Big Twin Lake near road; north margin of Big Twin Lake; near road along north shore of Big Twin Lake; northwest margin of Small Twin Lake near road; south margin of Hospital Lake; 3 to 5 m elevation; south of Arroyo Grande, San Luis Obispo County. The first three pairs of coordinates are ours from August 2017. At the first pair of coordinates we observed ~75 La Graciosa thistles in the dry lake bed in an area 15 x 61 m. At the second pair of coordinates we observed ~100 plants in the dry lake bed in an area 6 x 61 m, and Roddick (pers. comm. 2018) observed 186 plants here in 2018. At the third pair of coordinates we observed ~70 plants on slightly higher ground in an area 3 x 30 m. The plants included many robust individuals with multiple seed heads and many vegetative individuals. Other plant species in the immediate vicinity included marsh baccharis and poison hemlock. Big Twin Lake and Small Twin Lake were mostly dry and the beds covered with a layer (0.3 to 0.9 m) of predominantly dead California bulrushes. We found no La Graciosa thistle in Small Twin Lake in 2017, and we did not search the south or west margins of Big Twin Lake. We observed the following threats: reduced water/lack of water (Kofron 2019), vegetation debris in the lake beds, and invasive species (bull thistle, poison hemlock, pampas grass *Cortaderia*). The fourth pair of coordinates is ours in 2018 where we observed a new location on the northwest margin of Small Twin Lake near the road with 41 La Graciosa thistles in a 10 x 10 m area. Madsen (pers. comm. 2018) stated that he drove a tractor though the area two years prior, and also through the areas with the first and second pairs of coordinates. The fifth pair of coordinates is a new location and the first for Hospital Lake where Roddick (pers. comm. 2018) observed ~150 plants (102 m^2) in 2018.

Smith collected several specimens of La Graciosa thistle at occurrence 11 in 1973: SD124435, RSA321771, UCR111222 and UCSB52716 (Consortium of California Herbaria 2017 in USFWS 2018). In addition, Howald (1981 in USFWS 2018) reported 1 to 10 plants at each of four locations in 1981. McLeod (1984 in USFWS 2018) observed 11 to 50 plants in moist sand along the northwest margin of Big Twin Lake in 1984. Hendrickson and Parikh (1988 in USFWS 2018) observed 20 flowering plants in open shade/no shade in marsh along the south margins of Big Twin Lake and Small Twin lake in 1988. The additional reported locations were near the following coordinates: 35.070784, -120.606630; 35.070250, -120.606651; 35.070013, -120.606337; 35.068825, -120.609804; 35.067876, -120.604711. Cooper (1967) and Friedman (1986 in USFWS 2018) reported the landowner regulates water levels in the lakes. We viewed aerial imagery of the area using Google Earth (dated April 2015) in April 2017, and it is in the backdunes of the Callender Dunes. Most of the private property (492 ha) appears to be in a natural state, with ~10% of the vegetation having been cleared. The property is zoned for agriculture. The location is 2 km inland. The landowner also owns the property with occurrence 10. The Land Conservancy of San Luis Obispo County has owned a conservation easement over Big Twin Lake/Small Twin Lake/Hospital Lake since 2000, which

was formerly owned by the California Coastal Conservancy and the Nature Conservancy since 1996. Therefore, this occurrence is protected. Seeds from this occurrence could be used to re-establish occurrence 14 at Pismo State Beach, which is likely extirpated.

Possible location. 35.067116, -120.608934; 3 m elevation; "White Lake?" of the Dune Lakes, San Luis Obispo County; precise location unknown. This possible location is based on specimens collected in 1958 by C.F. Smith, including SBBG93737, SBBG93738 and RSA518077. The location is "along dead end road about duck ponds at Dune Lake (White Lake?)" (Consortium of California Herbaria 2017 in USFWS 2018). The coordinates that we give are along the north shore of White Lake, which is near (300 m) the occurrence at Big Twin Lake. This possible location is on private property of Dune Lakes Limited, and the lake is under a conservation easement with the Land Conservancy of San Luis Obispo County.

Occurrence 12. 35.012410, -120.607890; 35.012622, -120.607293; 35.012493, -120.607079; 35.012346, -120.607159; 15 to 18 m elevation; Surprise Lake, Oceano Dunes State Vehicular Recreation Area, San Luis Obispo County. The first pair of coordinates are ours, and the others are based on Chesnut (1998 in USFWS 2018). This occurrence was first reported by Howald (1981 in USFWS 2018) who observed 11 to 50 plants in 1981. McLeod (1987 in USFWS 2018) saw 50 plants in 1987. Hendrickson (1990 in USFWS 2018) observed 29 plants in 1990 (14 plants in east group, 15 plants in west group) and reported poor/low reproduction. The plants at the east end of the lake were in open shade under arroyo willow, along with native coyote brush, rosilla *Helenium puberulum*, and stinging nettle *Urtica dioica*. The soil was sandy with a thin layer (2 to 3 cm) of organic material. The west group was at the edge of a willow thicket, with *Rubus*, Pacific poison oak and goldenrod *Solidago*. Chesnut saw 54 plants in 1997 and 7 plants in 1998. Elvin (2008 in USFWS 2018) observed 10 plants in 2008. MIG/TRA Environmental Sciences (2016 in USFWS 2018) reported 37 plants in 2013 and 1 plant in 2015. We observed 37 plants in 2016 and 65 plants in 2017 at the west end of the lake, and habitat disturbance by feral pigs and herbivory of the plants. On 21 March 2017 the lake was dry but with a wet substrate of organic debris (leaves, bark, etc.). La Graciosa thistle occupied 10 x 20 m^2 at the west edge of the lake, in sandy soil with a thin upper layer of vegetation debris. The biggest plants (largest 46 cm diameter) were in the open, and feral pigs had foraged in the area. The lake was densely forested with arroyo willow, and those in the outer ring appeared dead, which is consistent with a drop in the groundwater table (Holland et al. 1995 in USFWS 2018; Alley et al. 1999; Chipping, pers. comm. 2017). We identified lack of water, habitat alteration by the debris of dead fallen trees and feral pigs as threats. In October 2018 we recorded only 2 to 4 young emerging plants at the same location, and we subsequently began supplemental watering as an emergency rescue action. On 10 April 2019, we observed 14 plants but no surface water despite considerable rains during the wet season. We viewed aerial imagery of the location using Google Earth (dated July 2016) in April 2017, and it is in the vegetated backdunes near the northwest edge of the Guadalupe Dunes. The location is 3.2 km inland. We consider this occurrence protected because it is in a remote part of Oceano Dunes State Vehicular Recreation Area that is now closed to motorized use and where visitation by the general public is unlikely (R. Glick, Oceano Dunes St. Vehicular Rec. Area, Calif., pers. comm. 2018). This occurrence is near extirpation.

Occurrence 13. 35.029879, -120.624003; 35.030135, -120.624759; 5 m elevation; northwest shore of Oso Flaco Lake, 166 m east of the outlet stream, Oceano Dunes State Vehicular Recreation Area, San Luis Obispo County. The coordinates are based on Hendrickson (1990 in USFWS 2018) and Chesnut (1998 in USFWS 2018). This occurrence was first reported by McLeod (1980 in USFWS 2018), but he subsequently saw no plants ("not found") in 1986. Hendrickson observed 34 plants in 1990 in two areas: "at the edge of a faint pathway among willows at the tip of the peninsula in open shade," and "about 175 m to the west in grass between some willows." She also observed off-highway vehicle damage to the habitat and poor/low reproduction. Chesnut saw no plants in 1998, and MIG/TRA Environmental Sciences (2016 in USFWS 2018) reported no plants in 2015. We saw no plants in 2018 during monitoring/removal of pampas grass, and we noted that the shrub vegetation surrounding the lake is becoming increasingly more dense. This occurrence is likely extirpated. Herbarium records include: RSA312493, UC1192200 and UCD130681 collected in 1949; CAS432751, GH427983 collected in 1960; CDA1569, CDA1570, CDA1571, CDA1572 and DS500655 collected in 1962; and OBI61046 collected in 1968 (Consortium of California Herbaria 2017 in USFWS 2018). We viewed aerial imagery of the area using Google Earth (dated April 2013) in April 2017, and it is in the vegetated backdunes at the south edge of the Callender Dunes. The location is 849 m inland. We consider this occurrence protected because it is in a part of Oceano Dunes State Vehicular Recreation Area that is now closed to motorized use, and visitation by the general public is unlikely (Glick, pers. comm. 2018).

Possible location. 35.034414, -120.630551?; 7 m elevation; blowouts in sand dunes toward the ocean on west side of Oso Flaco Lake, Oceano Dunes State Vehicular Recreation Area, San Luis Obispo County;

precise location unknown. This possible location is based on two herbarium specimens (JEPS24720, SBBG12577) collected in 1960. The coordinates that we give are imprecise and based on the vague information for the two specimens (Consortium of California Herbaria 2017 in USFWS 2018).

Occurrence 14. 35.107367, -120.625009; 7 m elevation; Pismo State Beach (4.25 km^2), northwest Oceano, San Luis Obispo County. This occurrence is based on an observation by G. Holstein (Zentner Planning and Ecology, Sacramento, Calif., pers. comm. 2017) in 1969, and the coordinates are his. He observed several La Graciosa thistles growing in sand on level ground in small openings in arroyo willow forest in a southeast corner of the park, northwest of the junction of Pier Avenue and Norswing Drive. Holstein stated that the openings were dominated by ice plant *Carpobrotus edulis*, which was crowding out La Graciosa thistle. He did not see the plant in 1976 (Hendrickson 1990 in USFWS 2018). McLeod (1986 in USFWS 2018) reported the occurrence as "undoubtedly extirpated." Hendrickson surveyed the ponds and associated wetlands near Pismo State Beach in 1990 and observed that iceplant was spreading over large areas. She saw no plants and likewise reported the occurrence as extirpated. Holstein visited the park again in 2016, and the basic geography had not changed substantially. We saw no plants in 2017. We viewed aerial imagery of the location using Google Earth (dated April 2015) in March 2017, and it is adjacent to Meadow Creek in a low terrace 900 m north of Arroyo Grande Creek (Cooper 1967). Although the park is mostly undeveloped, it is surrounded by urban development on three sides. The occurrence is 690 m inland. Herbarium specimen UC455492 was collected at "Oceano" in 1910 in "low grassy land among sand hills (Consortium of California Herbaria 2017 in USFWS 2018), and it is attributed to this occurrence (CDFW 2017). Although this occurrence is protected, it is likely extirpated. Seeds from occurrence 11 at Big Twin Lake, which is the nearest occurrence to the south, could be used to re-establish this occurrence.

Occurrence 16. 35.057423, -120.603537; 9 m elevation; Black Lake, San Luis Obispo County. The coordinates that we give are the center of Black Lake because precise details are lacking. Hoover (1970) reported La Graciosa thistle in "moist hollows among coastal dunes, at least from Black Lake southward." However, no herbarium specimens document this occurrence. Turner and Pemberton (1983 in USFWS 2018) reported La Graciosa thistle at Black Lake in 1983, but they subsequently corrected their identification to clustered thistle (Hendrickson 1990 in USFWS 2018). McLeod (1986 in USFWS 2018) and Hendrickson saw no La Graciosa thistle in 1986 and 1990, respectively. Hendrickson observed that much of the lake had dried and its bed was covered with green algae and nettle *Urtica*, but some potential habitat remained. The California Coastal Conservancy acquired the property (58 ha) with Black Lake in 1986 and subsequently transferred ownership to The Nature Conservancy, which transferred ownership to the Land Conservancy of San Luis Obispo County in 2000. Roddick (pers. comm. 2017, 2018) searched around the lake in 2017. She saw no La Graciosa thistle, very little habitat and abundant bulrushes, and she reported a lake depth of 2.4 m (3.5 m in 1975; Smith et al. 1976). We viewed aerial imagery of the location using Google Earth (dated July 2016) in April 2017, and it is in the backdunes of the Callender Dunes. The area is mostly undeveloped, but some vegetation clearing has occurred nearby to the southeast. The location is 2.3 km inland. Because this occurrence is on property of the Land Conservancy of San Luis Obispo County, we consider it protected. However, the occurrence is likely extirpated.

Occurrence 18. 34.977337, -120.620169; 34.975678, -120.617513; 34.976689, -120.615085; 34.977660, -120.615529; 34.981079, -120.615568; 10 to 23 m elevation; vicinity of monitoring wells L11, M-12A, N-12A (restored, outplanted), M-11 and M-2, Guadalupe Oil Field, 720 m north of Santa Maria River, 4.3 km northwest junction of State Highway 1 and State Highway 166 in Guadalupe, San Luis Obispo County. The coordinates are based on Padre Associates (2017 in USFWS 2018). The La Graciosa thistle are in five swales immediately adjacent to paved roads and oil pads, and in an area comprising 18 ha. This occurrence was first identified by Hendrickson (1990 in USFWS 2018), who observed 137 plants (20 m x 30 m area) in 1990. The plants were in moist sandy soil in a swale among stabilized dunes, with low herbaceous cover (no willows) including marsh baccharis, Pacific silverweed and yerba mansa. Lebednik (1995 in USFWS 2018) observed 31 plants in 1995 and disturbance by cattle grazing and gophers. Elvin (2006 in USFWS 2018) also observed cattle grazing on La Graciosa thistle in 2006. We have monitored this occurrence since 2006 (Padre Associates 2017 in USFWS 2018). We observed 913 individuals in 2017 and 748 individuals in 2018. From 2006 to 2018, the numbers of plants ranged from 240 individuals in 2010 to 23,590 individuals in 2014, with most of the latter number comprising offspring seedlings of outplanted individuals. We viewed aerial imagery of the area using Google Earth (dated July 2016) in April 2017, and it is in the backdunes of the Guadalupe Dunes. The location is 2.5 km inland. This occurrence is managed by Chevron Corporation, and it is currently protected because the oil field remediation and restoration activities are occurring under a USFWS (2005) biological opinion.

Occurrence 19. 34.967390, -120.592720; 17 m; immediately north of Santa Maria River in San Luis Obispo County, 2.2 km northwest junction of State Highway 1 and State Highway 166 in Guadalupe, Santa Barbara County. The coordinates are based on Ingamells (1991 in USFWS 2018). This occurrence was first reported by Ingamells, who estimated 100 plants in 1991. The plants were growing on irrigated grazing land with recycled water from the wastewater treatment plant of the City of Guadalupe. Associated plants were native balloon sack clover *Trifolium depauperatum*, invasive white clover *T. repens*, bull thistle, blessed milkthistle, and annual grasses. Ingamells reported the species to be thriving, likely due to improved hydrology from wastewater spray and reduced competition with annual grasses by grazing. This occurrence is on private property (158 ha), most of which has been disked irregularly and seeded with cattle fodder in recent years (pers. obs. 2017). The City of Guadalupe holds an easement over the area for discharge of water from its wastewater treatment plant. We visited the property with the landowner in August 2017, and it was mostly pasture. Robert Castellaños showed us all locations with water (wooded channel along north boundary, wet pasture on eastern part, lake and ditch along south boundary), but we saw no La Graciosa thistle. Invasive bull thistle was especially dense on the northwest part of the property. We identified agriculture and invasive species as threats. We viewed aerial imagery of the location using Google Earth (dated July 2016) in April 2017, and it is in the dry bed of the Santa Maria River. The property was used for cattle grazing in 2017 (300 head of cattle). It is 5.3 km inland. The occurrence is not protected, and it is likely extirpated.

Occurrence 20. 20A: 35.038460, -120.611143; 10 m elevation; in unnamed wooded swale, 749 m west of Jack Lake and 1.6 km west of ConocoPhillips Santa Maria Refinery, on Oceano Dunes State Vehicular Recreation Area, San Luis Obispo County. The coordinates are based on Hendrickson (1990 in USFWS 2018) and Chesnut (1998 in USFWS 2018). Hendrickson reported a new occurrence with 12 plants in a swale "0.5 mi W of Jack Lake," along with damage to the plants by rabbits in 1990. Chesnut saw no plants at this location in 1998. Heavy rainfall had inundated the location, and he surmised that plants may emerge after the area dried. MIG/TRA Environmental Sciences (in USFWS 2018) reported no plants in 2015. We visited the swale in 2017 and saw no plants. The swale is between tall, mostly vegetated and stabilized sand dunes. Further, the swale is densely forested with arroyo willow, and many of these were dead and collapsed, which is consistent with a drop in the groundwater table (Holland et al. 1995 in USFWS 2018; Alley et al. 1999; Chipping, pers. comm. 2017). The swale held no water but the soil was damp. In brief, the area indicated by Chesnut is now likely altered by the debris of fallen trees. Although we searched much of the swale in 2017, a large portion was not searched, which presents the possibility the plant was present but not observed. However, La Graciosa thistle at this location is likely extirpated. The ecosystem is mostly intact and in a natural state, with exception of lack of water. Efforts are underway to reduce perennial veldt grass *Ehrharta calycina* in the landscape by spraying with herbicide. We identified lack of water and habitat alteration by fallen trees as threats in 2017. We viewed aerial imagery of the location using Google Earth (dated April 2013) in March 2017, and it is in the backdunes of the Callender Dunes. The location is 2 km inland. We consider this location protected because it is in a remote part of Oceano Dunes State Vehicular Recreation Area that is closed to motorized use, and visitation by the public is unlikely (Glick, pers. comm. 2018). 20B: 35.035720, -120.610308; 12 m elevation; Lettuce Lake, Oceano Dunes State Vehicular Recreation Area, San Luis Obispo County. The coordinates are for the center of the forested part of the lake, which is 313 m south of the swale mentioned above. McCoy (1980) reported La Graciosa thistle at Lettuce Lake and also reported substantial damage to the area by off road vehicles. Chesnut saw no plants in 1998. We saw no plants in October 2017, and the lake was vegetated with arroyo willow (thriving, not dead or fallen), but no surface water was present. This record is not validated by a herbarium specimen, and there are no additional reports for La Graciosa thistle at Lettuce Lake. The location is 2 km inland. We viewed aerial imagery of the location using Google Earth (dated April 2016) on May 2017, and it is in the backdunes of the Callender Dunes. The lake is 78 m from intensive row crop agriculture to the east. We consider this location protected because it is in a remote part of Oceano Dunes State Vehicular Recreation Area that is now closed to motorized use, and visitation by the public is unlikely (Glick, pers. comm. 2018). La Graciosa thistle at this location is likely extirpated.

Occurrence 28. 34.957544, -120.582722; 23 m elevation; roadside, West Main Street, 800 m west of junction with State Highway 1, south Guadalupe, Santa Barbara County (Consortium of California Herbaria 2017 in USFWS 2018). This occurrence is based solely on one specimen (UCSB47943) that was collected in 1983 (Consortium of California Herbaria). The specimen label states uncommon along roadside on sandy to loamy soil, next to a wet grazed pasture, with non-native perennial rye grass *Lolium perenne* and ripgut brome. Mark Elvin (USFWS, Ventura, Calif., pers. comm. 2017) saw no plants in 2005 and 2013, and we saw no plants in 2017. The road is now paved and with a drainage ditch along its south side. On the north

side of the road is a dense housing development, and to the south are disked agricultural fields and a nearby storage area for farm vehicles. The occurrence is now surrounded by intensive row crop agriculture and urban development, and it is likely extirpated. We identified agriculture, development, and road and ditch maintenance as threats in 2017. We viewed aerial imagery of the location using Google Earth (dated July 2016) in March 2017, and it is 1.2 km southwest of the Santa Maria River and in its south floodplain. The location is 6.3 km inland.

Occurrence 30. 35.019253, -120.626641; 13 m elevation; in unnamed depression, 946 m southwest of Oso Flaco Lake, Oceano Dunes State Vehicular Recreation Area, San Luis Obispo County. The coordinates that we give are at the lowest point of the depression. This occurrence is based solely on one specimen (UCSB22159) collected in 1967 that is in the herbarium at the University of California Santa Barbara (Consortium of California Herbaria 2017 in USFWS 2018; CDFW 2017). The plant was growing in sandy soil at "1000 yards southwest of Oso Flaco Lake." Chesnut (1998 in USFWS 2018) did not look for this occurrence because it was not recognized in 1998. We viewed aerial imagery of the area using Google Earth (dated July 2016) in March 2017. Although the precise location was not stated, the most likely place is a depression at our stated coordinates. The location is 833 m inland, and it is in the backdunes of the Guadalupe Dunes. Although the area appeared to be in a natural state, many vehicle tracks were visible across the depression. A controlled burn was conducted here in 2009, along with weed treatment. We saw no La Graciosa thistle in 2017. The conditions here were not good, with only a few places where the plant could exist. We consider this occurrence protected because it is in a remote part of Oceano Dunes State Vehicular Recreation Area that is now closed to motorized use, and visitation by the public is unlikely (Glick, pers. comm. 2018). Also, we recommend searching for La Graciosa thistle at the following coordinates, which are nearby places with lower elevations: 35.019649, -120.631349, 7 m elevation, at 432 m west of the first coordinates; and 35.017277, -120.623793, 6 m elevation, at 342 m southeast of the first coordinates.

Occurrence 31. 34.996673, -120.602917; 34.99620, -120.601750; 34.996795, -120.60290; 17 m elevation (lowest in valley); 3 Pond West, Guadalupe-Nipomo Dunes National Wildlife Refuge, San Luis Obispo County. The first two pairs of coordinates are ours, the third is from K. Scarazzo (USFWS, Ventura, Calif., pers. comm.). This occurrence was first reported by Chesnut (1998 in USFWS 2018) who observed 87 plants in 1997. In 2007, Elvin (2010 in USFWS 2018) observed ~50 plants, and many had been trampled, crushed and broken by cattle that wandered onto the refuge. As of 2010, a boundary fence had been installed and cattle eliminated. In 2010 (USFWS 2016), 300 La Graciosa thistles were observed in a 1 ha area; in 2013, 172 individuals; and in 2014, 10 individuals. In September 2017, we observed at least five La Graciosa thistles with seed heads near the center of a swale (second pair of coordinates) in an open area (5 x 10 m) in sandy soil with 1 cm of organic soil on top and a thin layer of vegetation debris. Multiple other post-flowering thistle plants were present but not identifiable to species because of decomposition or herbivory by rabbits. Other plants in the immediate vicinity were rushes, coyote brush and poison hemlock. Willows occupied several parts of the swale, and many appeared dead with some fallen. We saw no La Graciosa thistle in the fenced exclosure at the northwest end where plants previously occurred (first pair of coordinates). However, in September 2018, we saw three postflowering individuals in the fenced exclosure and two postflowering individuals at the second pair of coordinates. We saw no vegetative La Graciosa thistle in 2017 or 2018. The swale was dry with exception of a drying waterhole (likely excavated) at the northwest end of 3 Pond West in 2017 and 2018. On 22 March 2019, D. Kirkland (USFWS, Ventura, Calif., pers. comm.) observed 25 individuals in the fenced exclosure, and no surface water despite substantial rains during the wet season. Further, she reported that the majority of large trees were dead or dying. At least one herbarium specimen (UCR215242; Consortium of California Herbaria 2017 in USFWS 2018) documents this occurrence. 3 Pond West is in the backdunes of the Guadalupe Dunes, and it is 3.5 km inland. Although this occurrence is protected because it is on a national wildlife refuge, it is in decline and near extirpation. We identified lack of water and herbivory by rabbits as threats. As an emergency action, we recommend that temporary fencing be placed around any groups of La Graciosa thistle outside the exclosure to prevent herbivory by mammals. In addition, based upon similar landscape features seen in aerial imagery in Google Earth (imagery dated July 2016), we recommend that searches be conducted in the valley immediately to the north, which is also on the refuge, and especially in wetland locations at 17 m elevation. In 2014, Elvin (pers. comm. 2017) collected seeds from 3 Pond West and dispersed them on the refuge at Colorado Pond (34.994225, -120.602520; 34.993147, -120.600274) to the south and Myrtle Pond (35.014158, -120.634629) to the northwest. Searches at Colorado Pond in 2017 and 2018, and at Myrtle Pond in 2019 (K. Scarazzo, pers. comm.), yielded no La Graciosa thistle. Colorado Pond is in the back dunes, and Myrtle Pond is immediately behind the foredunes.

Occurrence 32. 34.979440, -120.598600; 22 m elevation; Entrance Ponds, 940 m northwest of junction of Thornberry Road with entrance gate to the Guadalupe Oil Field, San Luis Obispo County (Fig. 3). The coordinates are from Elvin (2006 in USFWS 2018), who visited the occurrence in 2006 and reported the plants being adversely affected by cattle grazing and trampling. The wetland comprises two swales, one that is on private property of Chevron Corporation (WR1-01) and one on the adjoining private property (WR1-02). A boundary fence extends through the wetland. The plants on the adjacent private property are in a dense willow woodland (pers. obs. 2017). We identified this occurrence in 2005, and we have monitored it annually. La Graciosa thistle rapidly declined here from 420 plants in 2005 to 0 plants in 2015, 2016, 2017 and 2018. This occurrence is now likely extirpated because of a decline in the groundwater table: 16.73 m above sea level in October 2001, 11. 87 m above sea level in October 2018; our data, Fig. 3). We viewed aerial imagery of the location using Google Earth (dated 9 July 2016) in May 2017. The wetland is immediately north of the paved road, and the general area appeared to be in a natural state. The wetland is in the backdunes of the Guadalupe Dunes and 4.4 km inland. We identified the threats in 2017 as wetland drying, groundwater decline, drought and invasive plants (Italian plumeless thistle, ripgut brome, foxtail fescue *Vulpia myuros*). In addition, we observed many fallen dead arroyo willow that were altering the habitat. The fallen dead arroyo willow are consistent with a drop in the groundwater table (Holland et al. 1995 in USFWS 2018; Alley et al. 1999; Chipping, pers. comm. 2017). The part of this occurrence on property of Chevron Corporation is currently protected because the oil field remediation and restoration activities are occurring under a USFWS (2005) biological opinion. However, the part of the occurrence on the adjacent private property is not protected. This occurrence is not documented by a herbarium specimen.

Occurrence 33 (new). precise location unknown; ~34.748658, -120.259412; 190 m elevation; marsh on Price Canyon Road, Price Ranch, 1.6 km northeast of Los Alamos, Solomon Hills, Santa Barbara County. This new occurrence is based on multiple herbarium specimens collected in 1973 and 1975 by C.F. Smith and J. Sainz, including OBI71274, OBI171305, SBBG65980, SBBG65981, SBBG65982, SBBG65983, SBBG80937, SBBG81838 and SBBG96310 (Consortium of California Herbaria 2017 in USFWS 2018). Smith (1976) included this occurrence with La Graciosa thistle, however, he stated it might represent an undescribed taxon. We viewed photos of the two specimens to which Smith specifically referred, and the herbarium labels state the following: "several in marsh about willows on Price Canyon Road northeast of Los Alamos..." in oak woodland, and "few plants 2-5 ft., scattered about edge of willows...in marsh on Price Canyon Road northeast of Los Alamos." After further study, the two specimens bear annotated labels with identification by D. Keil (2012 in USFWS 2018) of California Polytechnic State University. Keil determined them to be La Graciosa thistle, although "an unusual form, perhaps derived from hybridization with undetermined second species." The coordinates that we give are imprecise and based on locality data with the aforementioned specimens, along with viewing aerial imagery of the landscape with Google Earth. In addition, herbarium specimen RSA355126 is spotted water hemlock *Cicuta maculata* var. *bolanderi*, and it was collected also by Smith on one of the same dates in 1973. It is from a "freshwater marsh about willows...in valley oak (*Quercus lobata*) woodland north-east of Los Alamos, about 1 mile airline" (Consortium of California Herbaria). We viewed aerial imagery of the area using Google Earth (dated January 2015) in May 2017, and we identified the relevant property as Price Ranch by a prominent woodland in a water course in a valley. The property is mostly undeveloped and used for cattle grazing, while many of the adjacent and nearby properties are vineyards. The location is 32 km inland, and 8.5 km southeast of occurrence 2. It is in the headwaters of San Antonio Creek watershed. This occurrence is on private property (219 ha) and not protected. We were not able to obtain permission to visit this occurrence in 2017, so the current status is unknown.

Occurrence 34 (new). 34.973586, -120.608135 (Elvin, pers. comm. 2017); 13 m elevation. This occurrence is based on Elvin (2006 in USFWS 2018) who in 2006 observed <10 individuals at the edge of the Santa Maria River, 3.7 km inland on private property of Chevron Corporation in San Luis Obispo County. The location is 720 m southwest of the nearest group of La Graciosa in occurrence 18. Herbarium specimen CDA1576 (California Department of Food and Agriculture) was collected in 1974 in bottomland of the Santa Maria River, north of West Main Street, and 4 km inland in Santa Barbara County (Fuller 1975 in USFWS 2018). This particular area has a history of intensive row crop farming, which puts La Graciosa thistle at risk in this location (34.971398, -120.605113; 13 m elevation) on another private property. Although vague and imprecise, we attribute this latter location to occurrence 34. It is 367 m southwest of the location reported by Elvin (pers. comm. 2017). We visited the occurrence in May 2018 and saw no La Graciosa thistle. The area was characterized by tall annual grasses and other invasive species (blessed milkthistle, bull thistle, Italian plumeless thistle), a fence and grazing cattle. The part of the occurrence on the property of Chevron Corporation is currently protected.

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Cover: Photograph of YOY giant sea bass exhibiting "resting" behavior in a sand depression in the shallow area off the beach near Veteran's Park, Redondo Beach, CA. Photo with permission by Michael C. Couffer.